Desk P.R.R.

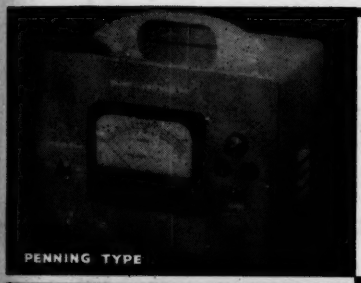
NATURE

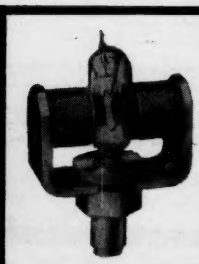
A Weekly Journal of Science

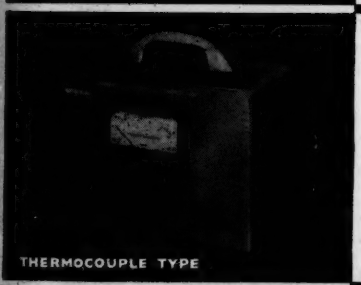
THE UNIVERSITY OF MICHIGAN
JUN 10 1959
PERIODICAL READING ROOM

Vol. 183, No. 4673 SATURDAY, MAY 23, 1959 2s. 6d.

METROVAC VACUUM GAUGES


PENNING TYPE

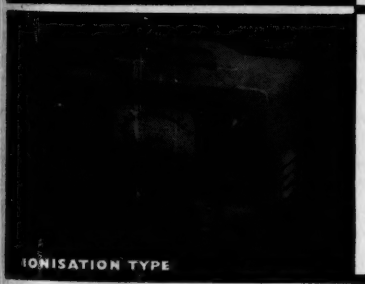


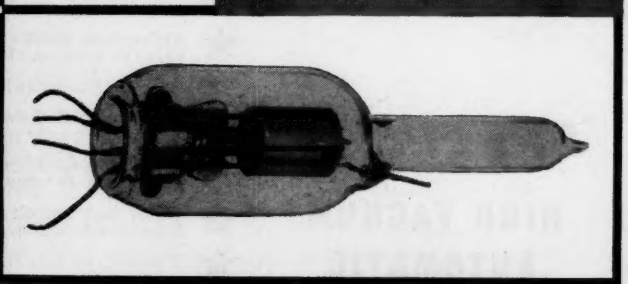

THERMOCOUPLE TYPE

For gas pressure measurement

from 1.0 mm. Hg. to 10^{-7} mm. Hg.

In addition to the control units and gauge heads illustrated, discharge tubes and Pirani gauges are also available and new units are constantly being developed.


IONISATION TYPE



The range includes instruments for:
ELECTRON MICROSCOPY
MASS SPECTROMETRY
CRYSTALLOGRAPHY
RADIOGRAPHY
MICRO-ANALYSIS
CRACK DETECTION
MATERIALS IRRADIATION
HIGH VACUUM ENGINEERING

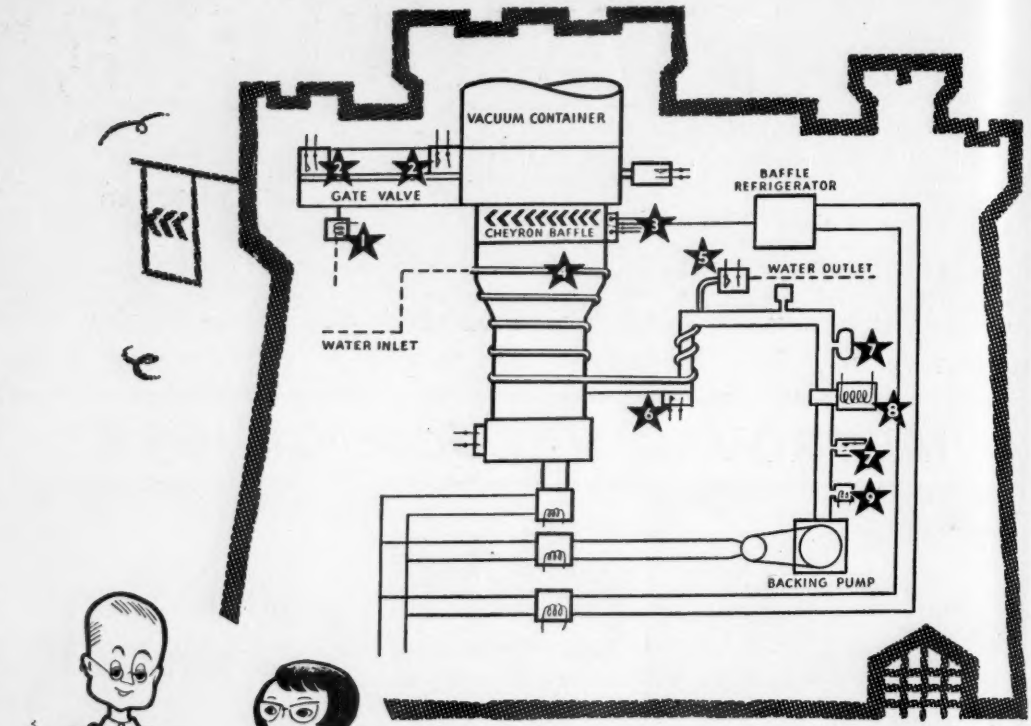
Please write for full details

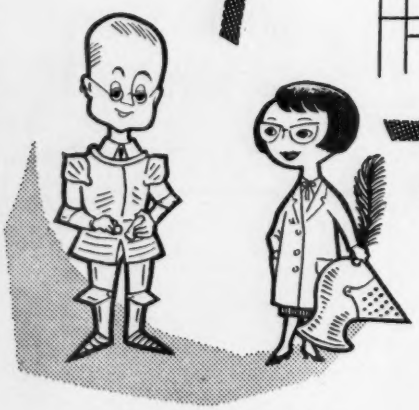
METROPOLITAN-VICKERS
ELECTRICAL CO LTD · TRAFFORD PARK · MANCHESTER, 17

An A.E.I. Company

N/R803

An advertisement in the Edwards "Mike Ron" and "Milly Torr" series.





"Guard duty's a bit old fashioned, Mike Ron. Edwards have made it all automatic!"

Milly Torr moves with the times. She is well aware that for many applications of a vacuum system the more the human element can be eliminated the better. Milly Torr, in common with an increasing number of vacuum users, finds that Edwards are with her all the way in this and offer a range of "Speedivac" devices that ensure the most imperturbable self-control from vacuum plant, leaving the operator to give full attention to the actual processing or technique for which the high vacuum is being employed.
Some of these "Speedivac" devices are indicated in the diagram above. Their functions are:—

"SPEEDIVAC" (Trade Mark)

HIGH VACUUM AUTOMATIC CONTROLS

1. Air control valve on line to compressed air operated gate valve to vaouum chamber or system.
2. Micro indicator switches controlling gate valve.
3. Thermal switch determines that temperature conditions are right for gate valve to be open.
4. Thermal switch prohibits opening of gate valve unless cooling water temperature is correct.
5. Water flow switch protects against flow rate fluctuations beyond safe limits.
6. Thermal switch indicating that pump fluid temperature is correct.
7. Vacuum switch pre-set to required pressure.
8. Magnetic valve for isolating backing pump.
9. Magnetic air admittance valve prevents rotary pump oil sucking back.

EDWARDS HIGH VACUUM LTD., MANOR ROYAL, CRAWLEY, SUSSEX, ENGLAND. Crawley 1500

MR11

NATURE

No. 4673 SATURDAY, MAY 23, 1959 Vol. 183

CONTENTS

	Page
British Books and Information Services Overseas	1417
Developments in Adult Education. By T. H. Hawkins	1419
The Psychopathology of Science. By Dr. A. R. Hall	1419
The Pinnipedia. By Prof. V. C. Wynne-Edwards	1420
British Water Beetles of the Family Hydrophilidae. By Miss Dorothy J. Jackson	1420
The Elusive Particle. By Prof. H. W. B. Skinner, F.R.S.	1421
Legendre Functions. By Prof. T. A. A. Broadbent	1421
Avian Folklore. By M. C. Burkitt	1422
Research on Hormones. By Prof. F. G. Young, F.R.S.	1422
Microscopy with X-Rays. By Dr. V. E. Cosslett	1423
Heterogeneity in Deoxyribonucleic Acids. By Dr. Julius Marmur and Prof. Paul Doty; Dr. Noboru Sueoka, Dr. Julius Marmur and Prof. Paul Doty	1427
News and Views	1432
Joachim Junge (1587–1657). By Dr. Douglas Guthrie	1435
Education in the Age of Science	1436
Public Libraries in England and Wales	1437
The Nature of Coal. By Dr. J. W. Whitaker	1438
Role of Poly-β-hydroxybutyric Acid in the Assimilation of Organic Carbon by Bacteria. By Prof. M. Doudoroff and Prof. R. Y. Stanier	1440
A New Short-acting Thiobarbiturate. By Dr. M. E. Peel, Barbara Macauley, Dr. H. O. J. Collier, Dr. J. H. Hunt, Dr. F. A. Robinson and Dr. E. P. Taylor	1443
Amino-acid Composition of the Proteins of Barley Leaves in Relation to the Mineral Nutrition and Age of Plants. By Dr. B. P. Pleshkov and Dr. L. Fowden	1445

(*Continued overleaf*)

An

INFRARED DETECTOR

sensitive from Visible to Microwaves

The operation of the Golay Detector is based upon the expansion of a gas of low thermal conductivity in contact with a non-selective heat absorbing membrane. The cell in which the gas (normally Xenon) is sealed has at one end a mirror membrane operating in a photoelectric amplifier system in such a way that the light intensity at the photocell varies directly with the gas expansion.

One of the major advantages of this system is a uniform sensitivity over a wide range of wavelengths. With suitable window material the Detector can be used to measure radiation intensities from visible light out to the microwave region. The adjoining table shows recommended window materials for specific wavelength regions.

The Detector can be delivered from stock with the following windows — KBr, Quartz, KRS-5, CsBr.

We shall be pleased to quote for alternative windows, for the Detector alone or for the full equipment including electronic and chopper units.

TABLE OF WINDOW MATERIALS

Material	Approximate useful wavelength limits
KBr	from visible to 26 μ — 27 μ.
CsBr	,, ,, ,, 35 μ — 40 μ.
CsI	,, ,, ,, 45 μ — 50 μ.
Quartz (0.25 mm thick)	,, ,, ,, 3.5 μ and from 45-50 μ to microwaves.
*Arsenic trisulphide	,, ,, ,, 10 μ.
*KRS-5	,, ,, ,, 35 μ.

*These two salts are non-hygroscopic but introduce some energy losses.

UNICAM

GOLAY INFRARED DETECTOR

UNICAM INSTRUMENTS LTD · ARBURY WORKS · CAMBRIDGE

U16

LETTERS TO THE EDITORS

Page

RADIOBIOLOGY

Uptake of Strontium by Pasture Plants and its Possible Significance in Relation to the Fall-out of Strontium-90.—Dr. P. B. Vose and Dr. H. V. Koontz 1447

Plants and Fall-out.—F. R. Fosberg . . . 1448

Comparative Sensitivity to Radiation of Seeds from a Wild Plant grown on Uraniferous and Non-Uraniferous Soils.—Dr. D. J. Mewissen, J. Damblon and Prof. Z. M. Bacq . . . 1449

Modification of the Oxygen Effect when Bacteria are given Large Pulses of Radiation.—D. L. Dewey and Dr. J. W. Boag 1450

Action of Ultra-violet Light on Skin: Effect of the Histamine Liberator 48/80 and Methotrimeprazine.—Prof. S. Claesson, G. Wettermark and L. Juhlin 1451

CYTOLOGY and GENETICS

Colour-blindness in Klinefelter's Syndrome.—Prof. Curt Stern 1452

Two Types of Ribonucleoprotein in the Nucleolus of Mammalian Cells.—Dr. Robert Love and Dr. T. P. Bharadwaj 1453

Size Relationship of Oocytes and their Nuclei in Two Species of Ascidians.—S. R. Venkatasubba Rao 1454

Effect of Colchicine on the Utilization of Thymidine labelled with Tritium during Chromosomal Reproduction.—L. F. La Cour, M.B.E., and Dr. S. R. Pelc 1455

BIOLOGY

Observations by Electron Microscopy on the Structure of an Acephaline Gregarine (*Apolocystis elongata*, Phillips and MacKinnon).—Dr. D. Lacy and H. B. Miles 1456

Pogonophora in the Hardangerfjord, Western Norway.—Prof. Hans Brattström . . . 1458

Utilization of Nutritive Spheres in the *Proteus* Group of *Amoebae*.—Sister Monica Taylor . 1458

Second Flowering in *Oryza sativa* (var. *indica*).—Subodh Kumar Roy 1458

Control of Leaf Senescence by Auxins.—Daphne J. Osborne 1459

A New Nitrogen-fixing Micro-organism producing a Red Pigment.—Gy. Németh 1460

Preparation of Autoradiograms for Electron Microscopy.—R. T. O'Brien and L. A. George, II 1461

PHYSIOLOGY and BIOCHEMISTRY

Use of 'Tremorine' for screening Anti-Parkinsonian Drugs.—E. M. Trautner and S. Gershon . 1462

Excessive Uric Acid Production in the Human induced by 2-Ethylamino-1,3,4-thiadiazole.—Dr. J. E. Seegmiller, Arthur I. Grayzel and Lois Liddle 1463

Page

Enzyme Activity in the 'Isolated' Colon.—Dr. B. F. Martin 1464

Distribution of Haptoglobins in Native Venezuelans.—Dr. M. L. Gallango de Rodríguez and Dr. Tulio Arends 1465

Pathways and the Physiological Site of Anserine Formation.—Prof. Theodore Winnick and R. E. Winnick 1466

A New Hæmagglutinating Substance in the Gm System, Anti-Gm.—Dr. Morten Harboe . . 1468

Exploratory Physicochemical Studies on the Sense of Taste.—Dr. Aetius R. Lawrence and Lloyd N. Ferguson 1469

Energy Compensation in the Crabtree Effect with Ehrlich Ascites Carcinoma Cells.—K. H. Ibsen, E. L. Coe and Dr. R. W. McKee . . . 1471

PATHOLOGY

Homologous Species Neoplastic Antibodies.—Dr. Hoy A. Cranston and Frederick O. Eggert . 1471

PSYCHOLOGY

The Water–Salt Receptor and Preference in the Rat.—Dr. J. A. Deutsch and A. D. Jones . 1472

PALÆONTOLOGY

Precambrian Coelenterata from Australia, Africa and England.—Dr. M. F. Glaessner . . 1472

CHEMISTRY

Some Alkyl-Aryl Sodium Sulphonates as Standard Substances in Detergent Chemistry.—E. Q. Laws and W. Hancock 1473

Labelling of Radiographic Contrast Media with Iodine-131.—J. Liebster, J. Kácl and A. Babický 1474

Infra-red and the Near Ultra-violet Absorption Spectra of Polyphenyl Derivatives of the Elements of Groups IV*b* and V*b*.—C. N. R. Rao, J. Ramachandran, M. S. C. Iah, S. Somasekhara and T. V. Rajakumar . . 1475

Binding of Water in Ferric Hydrate.—Prof. O. Glemser 1476

PHYSICAL SCIENCES

Geophysical Effects of High-Altitude Nuclear Explosions.—T. Obayashi, S. C. Coroniti and E. T. Pierce 1476

Auroral Isochasms.—Dr. Bengt Hultqvist . . 1478

Possible Causes of Geomagnetic Fluctuations having a 6-Sec. Period.—H. J. Duffus, J. A. Shand and Sir Charles Wright, K.C.B., O.B.E. 1479

Origin of Sodium and Lithium in the Upper Atmosphere.—Dr. T. M. Donahue . . . 1480

Effect of Cathode Inclusions on Electrical Discharges.—P. C. L. Pfeil and L. B. Griffiths . 1481

X-ray Spectrographic Determination of Strontium.—J. T. Campbell and H. I. Shalgosky . 1481

DIFCO *microbiological reagents and media*

TISSUE CULTURE and VIRUS PROPAGATION

These reagents are prepared and standardized to preserve unaltered the properties of the original material and include those commonly employed for the slide, roller tube and flask culture techniques for propagation and study of tissue cells and viruses *in vitro*.

REAGENTS OF ANIMAL ORIGIN—Desiccated and Liquid
Plasma, Sera and Serous Fluids, Embryos and Embryo Extracts, Ultrafiltrates.

REAGENTS, CHEMICALLY DEFINED—Dilute and Concentrate
Synthetic Media—Eagle-HeLa, Eagle L, Scherer, 199, 703 and all formulas. Balanced Salt Solutions—Earle, Gey, Hanks, Osgood, Simms, Tyrode and all formulas.

ENZYMES · INDICATORS · AMINO ACIDS · HYDROLYSATES
MEDIA ENRICHMENTS · BIOCHEMICALS · CARBOHYDRATES

Over 60 years' experience assures
UNIFORMITY : STABILITY : ECONOMY

Difco Laboratories

Prompt delivery from U.K. stock. Write for Difco Manual and technical leaflets to the sole agents:

BAIRD & TATLOCK (LONDON) LTD., CHADWELL HEATH, ESSEX, ENGLAND
Branches in London, Manchester and Glasgow *Agents throughout U.K. and all over the world*

T/ S/D02

Just Published —

Radio Studies of the Universe

by two young Jodrell Bank Scientists

R. D. DAVIES & H. P. PALMER

"Well written and illustrated, the book has a delightful absence of any theorizing, giving instead a clear account of the rapid progress in this field, and the glittering prospects ahead."
Times Literary Supplement.

Fully illustrated 25s. net

Published by
ROUTLEDGE & KEGAN PAUL

TECHNE TEMPUNIT

The Techne Tempunit is a self-contained constant temperature control for fitting to any vessel.

★ Indicating Thermostat.

★ Patented pneumatic control.

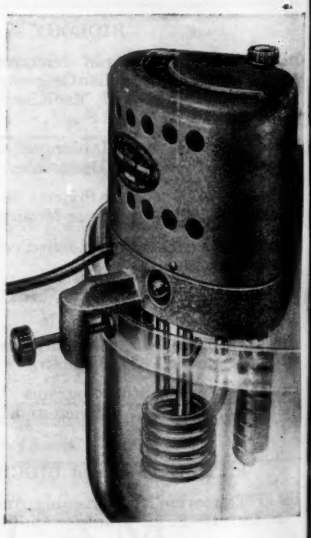
★ Accurate within ±0.05°C. in unlagged 4 gallon bath over the range. room temperature to 95°C.

★ Brushless silent motor.

★ 500w. or 1000w. heater.

★ Efficient stirrer.

★ Available for 200/250v., 50 cycle a.c. or 100/120v., 50/60 cycle a.c. supply.



TECAM

for full details write or telephone

TECHNE (CAMBRIDGE) LIMITED,
DUXFORD, CAMBRIDGE, ENGLAND.

Tel: Sawston 2246

BRITISH BOOKS AND INFORMATION SERVICES OVERSEAS

WHEN speaking in the debate on defence in the House of Commons on February 25, Mr. A. Head, a former Minister of Defence, said that his visits to South-East Asia, the Middle East and Africa in the past two years had increasingly convinced him that Britain is losing the battle for men's minds. It is, of course, as a contribution to this ideological warfare that President Eisenhower on March 13 asked Congress for 3,930 million dollars to continue the U.S. foreign aid programme, which includes 179 million dollars for technical assistance programmes in 1960 and 30 million dollars as a contribution to the technical co-operation and special projects programmes of the United Nations. An appreciable proportion of Colonial Development and Welfare Funds may be regarded as directed to the same end, and the stress laid on education at the Commonwealth Trade and Economic Conference at Montreal, and on information services, particularly on broadcasting and television, in the last annual report on the Colonial Territories, further indicates the growing realization of the importance of seeing that an accurate and objective picture of Britain is presented to the world.

It is against this background that the proposals outlined in the White Paper on Overseas Information Services* are to be considered. The plans outlined in the earlier White Paper of July 1957 led to an increase in expenditure on overseas information services, from £13 million to £15 million a year, chiefly through an increase in the number and strength of information posts, the re-deployment of external resources of the British Broadcasting Corporation, increased resources for the Central Office of Information, more television films, and a substantial expansion of the work of the British Council. Despite this expansion, details of which are appended to the new White Paper, annual reports of the British Council continue to testify to the inadequacy of the present effort, and in the last annual report Sir Charles Snow refers in particular to the unsatisfactory position of British books and other printed matter in the world overseas, including the Colonial territories. This has been the subject of persistent Parliamentary questions, and the travels of Dr. Charles Hill, Chancellor of the Duchy of Lancaster, to whom the survey of British information services was entrusted, have apparently shown him that British books are facing heavy competition in various areas, and convinced him that action is required. Nevertheless, in reply to Lord Stonehaven's request in the House of Lords on February 18 for a statement of the Government's plans for facilitating the increased distribution and sale overseas of British books and periodicals, particularly in countries where there are sterling restrictions, the Earl of Home could only state that Dr. Hill's study of these problems is well advanced and that a statement will be made as soon as possible.

On this question of books, the White Paper admits that there are countries where import restrictions hamper the British publisher in selling books at normal prices, although in these and other countries there are large numbers of students and others who cannot afford to pay anything like the ordinary economic prices for British books which they need or wish to read, and that other countries have done more than we have to devise ways and means of overcoming such obstacles. But it merely comments that the Government is studying ways of encouraging the flow of British publications overseas. Apart from this, the supply of books in existing British Council libraries is to be increased and more resources made available for the presentation of books to universities and other libraries. In addition, financial provision is being made for a pilot scheme of lending books to university and technical students in some countries for the duration of a course or session without the requirement to return the borrowed books in a fortnight or month. At present, Sir Charles Snow puts the total cost of British expenditure on the dissemination of the printed word at less than £500,000.

In replying in an adjournment debate on March 26 on the supply of British books and films overseas, Dr. C. Hill amplified the information given in the White Paper. There are ninety-nine British Council libraries, and help is given in maintaining about forty others. In the current year, £60,000 was spent on new books for these libraries; next year this will be increased to £171,000, and expenditure on periodicals will also increase. As regards exports of books, in 1937 the total value of books sold was £10·5 million and 30 per cent were exported; for 1946 these figures were £27 million and 24 per cent; and for 1957, £60 million and 37 per cent; in terms of individual books, probably about half the production is exported. Dr. Hill pointed out that the two British book export schemes have been closed down for political reasons in the countries concerned; he gave no further indication of the Government's intentions, though he re-affirmed that the study of the problem is almost complete. He pointed out that it need not be Government money which is blocked, and that Government action is not the only way of dealing with the problem. He regards the teaching of English and the provision of books, directly or through libraries, as the most important part of our long-term information work, and hopes for a general discussion before long on British Overseas Information Services as a whole.

The Government announced in the House of Commons on November 3, 1958, that a more intensive effort is required to ensure that British ideas, policies and objectives are widely known and understood, and the proposals outlined in the white Paper will increase the expenditure on overseas information services by a further £1·5 million. The expansion is designed particularly to meet changing conditions and techniques in parts of Asia and Africa,

*Overseas Information Services. Pp. 8. (Cmnd. 685.) (London: H.M. Stationery Office, 1959.) 6*d*. net.

particularly the demands arising out of the desire for education; although it should be pointed out that the White Paper gives scant attention to the possibilities of television, beyond observing that there is considerable scope for the establishment or development of television services in some Commonwealth and Colonial countries, and that much preliminary work is proceeding. Dr. Hill was questioned on this point in the House of Commons on March 16 and agreed as to the great and increasing demand which we are seeking to satisfy. The English language is in wide and growing use throughout the world as the language of education, administration, culture and technology, and it is this fact that gives such importance to the teaching of English, including the supply and placing of teachers, and the supply of the books and periodicals needed to sustain and increase its use. This is apart from the need to demonstrate continuously the merits of a free as compared with a totalitarian society, to show the British understanding of the aspirations of other nations and to counter hostile misrepresentations of our aims and achievements.

To do all this effectively requires, too, the maintenance of the reputation of British information services for accuracy and objectivity; but it should not be necessary to elaborate further the nature of these tasks to demonstrate how large a contribution this £16·5 million could make to the easing of the tasks of diplomacy and defence. As regards the British Council, the Government's proposals, which will increase the Council's grant to about £5 million, include the provision of more money to subsidize teaching posts, encourage exchanges of university teachers, offer more scholarships in Britain and provide special British Council officers to advise on problems in teaching English. The Council's work in Nigeria is to be strengthened, and provision made for exchange of visits and other activities in the educational and cultural fields with Czechoslovakia and Hungary.

On broadcasting, the growing listening public which local sound broadcasting stations command almost everywhere, and the increasing difficulty which short-wave transmissions from afar encounter in competing with medium-wave and very high-frequency programmes locally transmitted is emphasized, and the growing demand for recorded material on disk or tape for inclusion in local programmes is noted. To strengthen the signal of the Arabic, Somali and Swahili services, a new relay transmitter is to be completed at Berbera in the Somali Protectorate by the beginning of 1960, and the daily hours of transmission of the Arabic service of the B.B.C. are to be increased from 9·5 to 12 with increased coverage of events of local significance and greater local appeal in entertainment programmes. More help is to be offered to local broadcasting stations, particularly in eastern Africa and Aden, to which substantial grants have already been made from Colonial Development and Welfare Funds.

New posts are to be opened in the official information services and existing posts strengthened in New Zealand, South Africa and East Africa; but the main expansion of these official services will be in the production of material in the vernacular languages, as well as in English, for all Commonwealth countries, and in the improvement of communications. Special attention is being given to news items about people from overseas who are working or studying in the United Kingdom. More trade and technical periodicals will be distributed overseas, and the programme of visitors from overseas is again being expanded, especially for those from the Commonwealth.

The extent to which all this affects directly the work of the scientist and technologist is not very apparent from the White Paper itself, although it is obvious that measures to improve interchange, the flow of information—of the printed word and over the air—and to facilitate understanding and communication both within and without the Commonwealth must assist that increased scientific and technical co-operation for which the Duke of Edinburgh recently pleaded at Karachi. Moreover, the official services can never replace the work of the voluntary organizations, such as scientific societies. Some indication of the extent of present developments can, however, be gathered from the last annual report of the British Council, which during 1957–58 assisted more than 1,800 visits of scientific specialists to and from Britain—some 35 per cent of the total assisted. Indeed, of visitors to the United Kingdom in 1957–58 under the auspices of the British Council, 18·5 per cent were concerned with social studies, 42·6 per cent with science, 10·1 per cent with medicine, and 14·8 per cent with education, and the shift in subject pattern from the arts and humanities towards the pure and applied sciences is growing. British scientific books and periodicals form a considerable proportion of the Council's libraries, especially in those countries where interest in the sciences is high. While the scientist and technologist therefore will welcome the increased support to be given to overseas information services as indicated in the White Paper, the further statement of policy regarding measures to assist the flow of British books will be awaited with no less interest and even some anxiety. Until some measures have been taken to deal with the obstacles to the flow of British books abroad our economic and commercial interests, no less than those of science and technology, will continue to suffer, and British technology will not be effectively exported. Even such achievements as the nuclear reactor cannot be properly exploited if the relevant scientific and technical literature is not freely available. The full effect of at least some of the measures promised in this White Paper awaits action on the decisions to which it is to be hoped Dr. Hill's study will speedily lead him. Beyond this, however, the scientist or technologist who goes abroad should himself be among the best of Britain's interpreters, and possibly professional organizations and bodies responsible for young scientists and technologists going abroad for the first time could do more than at present to see that such recruits understand what is expected of them, the conditions they will meet and the equipment of mind and character as well as technique that they should take with them.

DEVELOPMENTS IN ADULT EDUCATION

Trends in English Adult Education

Edited by Prof. S. G. Raybould. Pp. xiii+258. (London: William Heinemann, Ltd., 1959.) 21*s*. net.

THE most remarkable feature of this book is that it should have been written at all. During the great debate of less than ten years ago some of the contributors to the book vociferously championed the pure white flag of liberal adult education and completely rejected all the mixtures which went under the false colours of extension classes, local education authority activities, informal pursuits, broadcasting and the like. Yet here is a book, more than half of which is concerned with trends quite unrelated to the traditional conception of adult education in Great Britain. It is a fine tribute both to the vigour of adult education itself and to the flexibility and resource of those who promote it.

With two exceptions, the contributors are all drawn from the department of adult education at the University of Leeds. They, and particularly the editor, deserve warm praise for setting down their recent experiences in such forthright, objective and yet provocative fashion; this applies no less to the two distinguished contributors from outside the University walls.

The great debate about the purpose and content of adult education was sparked off by Prof. S. G. Raybould himself in 1949. The issue, quite simply, was whether or not the time-hallowed name of adult education could be given to any activities unconnected with the tutorial classes which had been jointly developed by the Workers' Educational Association and university extra-mural departments. How the debate proceeded—and how other adult education activities developed apparently with the connivance of those who opposed them—is carefully set out in a well-written chapter by Roy Shaw. This, surely, should have been the starting point of the book.

The controversial place of the Workers' Educational Association in the adult education movement of to-day is carefully analysed by a great friend of the movement, J. F. C. Harrison. Regretfully he arrives at the only possible conclusion that the Association is but a shadow of its former self, has failed to adjust its aims and organization to the needs of 1959, and with bewilderment and frustration arising from lack of national leadership, stands in marked contrast to the buoyancy and optimism which marks the university adult education movement of to-day.

The latter is described by J. W. Saunders. Since 1945 the virtual monopoly of university services by the Workers' Educational Association has been challenged by education directly provided by the extra-mural departments of the universities, now recognized as 'responsible bodies' by the Ministry of Education. Besides traditional classes for the 'workers', extra-mural departments are providing an ever-increasing number of specially designed courses to appeal to specialized professional people and to students who have had the privilege of a secondary and even a university education; "new social purposes, new social fulfilments are being met". If, as Sir Charles Morris writes in the foreword to this book, "at all stages a great part of education must always be self-education", the university extension movement is surely right to give urgent attention to the quality and standard of people who emerge from schools and universities into a world of specialists.

Other chapters describe the increasing attention paid to adult education by local authorities; the direct contribution of broadcasting and, incidentally, the value of market research in giving the customer what he requires; the immeasurable value of residential adult education colleges by their most worthy exponent, Guy Hunter; the educational influences of organizations such as Women's Institutes, Townswomen's Guilds and various local societies; some experimental work in trade union education which needs to be followed up with much greater enthusiasm than appears to be the case; and finally, a review by Prof. Raybould of developments since the Ashby Report was published in 1954. Most of the recommendations set out in that Report have been implemented; their influence on adult education trends have been considerable. One recommendation made by the committee was that sooner or later adult education must fight for a due share of the finances of universities, instead of continuing to be a branch of university activity protected by ear-marked grants from the Ministry of Education. Prof. Raybould believes that the time to adopt this recommendation has arrived. His views are earnest and compelling and could well initiate another great debate.

T. H. HAWKINS

THE PSYCHOPATHOLOGY OF SCIENCE

The Sleepwalkers

A History of Man's Changing Vision of the Universe. By Arthur Koestler. Pp. 624. (London: Hutchinson and Co. (Publishers), Ltd., 1959.) 25*s*. net.

THE story of the substitution for the tight, cosy, Earth-centred world of Aristotle of the infinite, centreless, mechanistic universe of Newton is a drama which the dullest of historians could not conceal; it is told again by Mr. Koestler with all the stylistic verve of an experienced novelist and with adroit use of the materials and ideas pieced together by historians of science during the past two generations. Through detailed studies of Copernicus, Kepler and Galileo as examples of the medieval, the transitional and the 'terrifyingly modern' scientific mentality the author has succeeded brilliantly in giving the general reader a vivid account of what the change from medieval to modern cosmology involved. For this is the core of the book; nothing between Aristarchos and Copernicus holds much interest for Mr. Koestler, and his account of cosmology after Galileo is equally brief. Newton's austere mind commands his admiration, but not his imaginative sympathy.

Although he makes effective—not invariably quite accurate—use of recent work in the history of science, Mr. Koestler's opinions will not always commend themselves either to scholars or to scientists. He constantly under-rates Ptolemy's great achievement because he finds the theory of epicycles ridiculous, while recognizing that the epicycles were tools of mathematical analysis and not regarded as physical realities. Conversely, he argues that Galileo should have been satisfied with the status of the Copernican system as a "working hypothesis", since he could not prove its *de facto* truth. Yet the Church was only

prepared to admit Copernicanism as a calculating device, precisely like Ptolemy's epicycles. To overlook this fact and to under-estimate Galileo's concern with physical reality rather than with mathematical hypotheses of planetary motion is to run in peril of the "double-think" that Mr. Koestler detects in others, and contributes to that lack of feeling for Galileo which is so marked and astonishing a feature of this book.

For Mr. Koestler is a violent partisan in the way he treats ideas and characters. For him Copernicus is a fumbler, a second-rate second-hand Aristarchos, cold, timid, anxious; Galileo is arrogant, vain and deceitful, contemptuous of the achievements of others and rashly confident that he can over-reach the theologians, so precipitating a disastrous and unnecessary conflict. Kepler is the hero of the story because his scientific discoveries were the fruit of his mystical leanings; Galileo its villain because he turned his back on Kepler and constituted himself the champion of science against religion. All this forms an interesting and provocative point of view; but it is a highly personal one.

Not less singular is Mr. Koestler's chief term of historical appreciation: schizophrenia. The tension he finds in the history of cosmology he attributes to "split-mindedness" and its product "double-think". This journalistic distortion of a precise psychopathological concept may itself be deplorable to purists: it becomes objectionable to historians when the whole of the Middle Ages is dismissed as an age of the split mind, and to physicists when quantum mechanics enters the category of "double-think" (whatever that may mean). Perhaps one should not insist on an author's idiosyncrasy; in this book, however, psychological theorizing is fundamental to the interpretation of history, and to the conclusions of the epilogue on the contemporary state of scientific thought. In short, the brilliance of the book lies in the literary use of recent historiography; Mr. Koestler's allusions to the mental hospital give it an additional and perhaps more dubious colour.

A. R. Hall

THE PINNIPEDIA

Seals, Sea Lions and Walruses

A Review of the Pinnipedia. By Victor B. Scheffer. Pp. x+179+32 plates. (Stanford, Calif.: Stanford University Press; London: Oxford University Press, 1958.) 30*s*. net.

THE Pinnipedia are a highly specialized group of about thirty species, which present unusual difficulties to the systematist. Their large, greasy skins are difficult to preserve, and are consequently scarce in museum collections; the skeletal parts, especially skulls—on which the systematist has usually to rely for comparative material—in some cases undergo progressive change of form and proportion as the animal's age advances. Particularly in the various polygamous species the males do not stop growing at maturity, as do most warm-blooded vertebrates, but may continue to increase in size, in some species quite rapidly, throughout life. Consequently the average measurements of a sample can be materially affected by the age-composition of the group from which it was taken. In these circumstances the diagnosis of subspecies often proves almost impossible, and the boundaries between species and genera sometimes appear indefinite also. In the past, confusions of names have been common and synonyms are therefore unusually numerous.

No comprehensive synopsis of the Pinnipedia has appeared since the early years of the century, and this makes Dr. Scheffer's modern, practical and authoritative review of their taxonomy and distribution particularly welcome. I notice with interest that he feels the group ought to be regarded as an order in its own right, separate from the Carnivora. Fifteen of the twenty genera into which he divides the present-day forms are monotypic; among these is *Phoca*, to which only the common or harbour seal (*P. vitulina*) is here assigned. Seals are characteristically distributed coastwise, often strung out in long chains rather than occupying broad regions, and this may have facilitated speciation. In none of the five polytypic genera is there any instance of two species in the same genus (as defined by Scheffer) occurring together in the same area.

Some of the more widespread species have disrupted ranges: the harbour seal, for example, has two distinct North Atlantic centres, one on each side, and two corresponding ones in the Pacific. Here the author has decided to assign separate subspecific names, notwithstanding the fact that constant physical differences between the races are not always discernible. The name *mellonae* is applied to a fifth, freshwater population in Ungava, "not because it stands for a remarkably different kind of seal but because it represents a situation . . . ". Such an unorthodox criterion is presumably justified by the convenience of having labels for populations which are geographically distinct and definable as living entities.

The systematic section is prefaced by fifty pages of highly condensed, almost laconic information, chiefly on the anatomy, physiology and evolution of seals. The introduction contains an illuminating table in which the world populations of all the numerous forms are critically estimated; one recent race—the fur-seal of Juan Fernandez—is probably extinct, and some others are dangerously low; but considering the ravages that seals and their allies have suffered all over the world at the hands of man they have shown surprising powers of resistance and recovery.

The book contains distribution maps, excellent photographs and figures, and a very full bibliography. Only systematic names are listed in the index; but some indication of the numerous topics included in the book can be gleaned from the subheadings to the introductory chapters, given in the table of contents. It is a very useful work of reference.

V. C. Wynne-Edwards

BRITISH WATER BEETLES OF THE FAMILY HYDROPHILIDAE

British Water Beetles

Vol. 3. By Prof. Frank Balfour-Browne. Pp. by liii+210. (London: The Ray Society, 1958. Sold Bernard Quaritch, Ltd., 11 Grafton Street, W.1.) 30*s*.

PROF. F. BALFOUR-BROWNE is much to be congratulated on having produced a third volume on British water beetles. The first two volumes, published in 1940 and in 1950, deal with the Hydradephaga, the present volume with the aquatic

Hydrophilidae. The author writes with intimate knowledge of the species dealt with, acquired during his long experience as a field naturalist. As in the previous volumes, no descriptions are given of the species, and a successful identification is dependent on the correct interpretation of the keys, aided, however, by many useful figures illustrating anatomical features. An account of the life-history of the Hydrophilidae is included in the introduction, and many interesting biological notes are scattered throughout the book. More information on the larvæ and their habitats—where these are known—would have been of much value, but it is hoped that this book will induce others to study the biology of these interesting beetles, as Prof. Balfour-Browne has done so ably in the case of *Hydrobius fuscipes*, L.

The maps showing county and vice-county boundaries are a great improvement on those given in the earlier volumes, and are most useful in showing at a glance the known distribution of a species. Few errors have been noticed in the text, but no map is given of *Ochthebius minimus* Fab. though quoted on p. 160, and many authors are mentioned without inclusion in the bibliography. An interesting account is given of the male genitalia in some species of *Ochthebius*, *Hydræna* and *Hydrochus*. The author considers that the modifications described render the males of some species incapable of mating and he believes that in consequence parthenogenesis occurs. This is a problem requiring investigation.

Highly controversial are the author's views given under the heading "Distribution and Means of Transport", though what transport he envisages for flightless water beetles is not specified. His outlook on the problem of the present distribution of water beetles is dominated by his idea of recent "invasions" arriving not only at the coast from the Continent but accounting also for the occurrence of various local species, adapted to special habitats, in all parts of the country, such as *Helophorus tuberculatus* Gyll, in Lanark (p. 96). The occurrence of uncommon species in localities not previously or recently worked is no proof of their recent arrival, and especially is this true when the specimens collected are incapable of flight, as in the case cited (p. lii) of *Noterus crassicornis* Müll., a species which is common in a Fife loch where 90 specimens have been taken, all brachypterous. It is more likely that local aquatic species are the last remnants of a population that was formerly much more extensive, before drainage and cultivation had destroyed many habitats.

Argument is an excellent stimulus to further research, and Prof. Balfour-Browne has written a stimulating book which will arouse fresh interest in these beetles and prove of much value to the specialist.

DOROTHY J. JACKSON

THE ELUSIVE PARTICLE

The Neutrino

By Prof. James S. Allen. (Investigations in Physics, No. 5.) Pp. viii+168. (Princeton, N.J.: Princeton University Press; London: Oxford University Press, 1958.) 36*s*. net.

THIS small book gives an excellent description of experimental work on the neutrino, which only in the past few years has been shown to have any direct physical property apart from balancing energy, momentum and spin in β-decays. It is a tribute to modern methods of experiment that neutrino-capture in hydrogen has been demonstrated by Reines and Cowan in spite of the fact that a neutrino beam could traverse a thickness of solid material measured in *light years* without appreciable loss. The timing of the book was unlucky, because it was written before or during the revolution which occurred in the last half of 1957 and in 1958, due to the discovery of the non-conservation of parity in β-decay. Dr. Allen was therefore obliged to cover the changes in "Notes added in Proof". This he has done satisfactorily, but the subject has been so much changed very recently that a much clearer picture would be possible now.

Nevertheless the book is a useful one since it is concerned mainly with descriptions of experimental methods in the very difficult field of neutrino physics, and does not go into details of theories, some of which have changed.

The subject has had a curious history. Experimentalists were misled for many years by the conservation of parity theory, which was not based on any universally valid evidence, into not looking for such an easily detectable phenomenon as the polarization of electrons in β-decay. Some wrong results in the electron-neutrino angular correlation experiments, understandable in view of their difficulty, also contributed to the confusion. However, now everything seems in order on the two-component neutrino theory of Lee and Yang, both for β-decay and for pion decay. It has not happened often that so tricky a field of physics has been cleared up so quickly, as a result of brilliant theoretical work, which suggested many key experiments.

H. W. B. SKINNER

LEGENDRE FUNCTIONS

Fonctions Sphériques de Legendre et Fonctions Sphéroidales

Tome 2. Par Dr. Louis Robin. (Collection Technique et Scientifique du C.N.E.T.) Pp. viii+384. (Paris: Gauthier-Villars, 1958.) 5,300 francs.

THE second volume of Dr. Robin's comprehensive treatise deals with the associated Legendre functions $Pm_n(\mu)$, $Qm_n(\mu)$. The problem of defining these functions for unrestricted complex values of m, n and μ was tackled by several mathematicians in the late nineteenth century, and finally solved by Hobson (1896), who used for this purpose the double-circuit contour integrals devised by Jordan and Pochhammer. An essentially complete theory was expounded in full in Hobson's classical treatise on spherical harmonics (1931), and Dr. Robin has based his book very largely on this work, with some extensions and some elaboration of detail. There are three sections. In the first, which occupies more than half the book, the formal discussion of the functions is developed in exhaustive fashion. In the second, asymptotic formulæ and the Stieltjes-Hobson inequalities are considered; in the third, convergence and summability of series of Legendre functions. The influence of Hobson and of other British mathematicians, for example, Barnes, Watson, MacRobert, is very evident, and little of importance has escaped the author's attention. The book is for reference rather than for light reading, and for British readers is scarcely likely to replace Hobson; but Dr. Robin has taken account of work done since 1931, and has also incorporated earlier work, for example, on

Cesàro summability (C, k) with $k<\frac{1}{2}$, which is only briefly referred to by Hobson.

In 1908, Barnes gave an alternative to Hobson's definition, using a contour of integration parallel to the imaginary axis and a gamma-function integrand. Dr. Robin refers to this and also to Barnes's penetrating study of asymptotic expansions. But since Barnes's work has never been published in full, and since he claimed that his methods diminish the labour to about one-third of that required on Hobson's approach, it would be interesting to see what, for example, the first section of Dr. Robin's book would look like if written out on Barnes's lines. This, however, would in no way detract from the value of the present volume as an admirable work of reference.

T. A. A. Broadbent

AVIAN FOLKLORE

The Folklore of Birds

An Enquiry into the Origin and Distribution of some Magico-Religious Traditions. By Edward A. Armstrong. (The New Naturalist: a Survey of British Natural History.) Pp. xvi+272+33 plates. (London: William Collins, Sons and Co., Ltd., 1958.) 30*s*. net.

BIRDS have always fascinated humanity. Only of recent years has proper attention been paid to the importance of folklore for the study of history and of the way people thought in the past. It is only just in time. Folktales and folklore were seldom written down but were passed from generation to generation by word of mouth. Nowadays the spread of so-called civilization has meant that, except here and there in outlying districts, everything has been forgotten. Recently there has been a move to collect what has survived and to examine at the same time the folk legends of modern primitive peoples. Fortunately, although not written down, pictures recalling the folklore have often been used as decorative motifs in peasant crafts, and a close study of these has helped to elucidate the underlying legends. Of course, not every figure of a bird either in prehistoric times or at a later period illustrates some myth or legend. Indeed, I doubt whether the bird-sign on the top of a staff which can be seen in the Under Cave at Lascaux in connexion with the happy looking rhinoceros walking away from the disembowelled bison nearby is anything but the sign manual of some hunter concerned with the painting of the scene. This may well have been made for some definite purpose, though not necessarily to illustrate what we should to-day call folklore. Whether one can talk about folklore as such in palæolithic times one may be allowed to doubt, but in the later periods there is often documentary evidence which explains some of the art.

Edward A. Armstrong is well known as a naturalist. He has written much on bird life, and his book, "The Wren", is deservedly popular. In the present volume he starts with early times and continues with chapters devoted to various species of birds which figure especially in folklore tales. Thus we find chapters on the goose, the red-throated diver, the raven, the green woodpecker, the eagle, the wren, and a number of song birds. Each chapter describes some of the legends attached to the particular bird or group of birds. There is also an account of the beliefs which have grown up in various places, that the dead return as birds; for example, the story of the fisherman seeking his dead brother who had foretold that he would return as a gannet after death and would be recognizable by his black armsleeves. The author has thrown out his net widely. In time he ranges from prehistoric to recent periods, and geographically from Hudson's Bay to Zimbabwe in Rhodesia. There are numerous and delightful illustrations, some line blocks, some in half-tone and one in colour. The book as a whole is a scholarly account of the subject and a great deal of material has been collected. It also makes good reading. Archæologically and anthropologically it is a work which the purchaser will find well worth possessing.

M. C. Burkitt

RESEARCH ON HORMONES

Recent Progress in Hormone Research, Vol. 14

Proceedings of the Laurentian Hormone Conference 1957. Edited by Gregory Pincus. Pp. vi+582. (New York: Academic Press, Inc.; London: Academic Books, Ltd., 1958.) 13.50 dollars.

THE fourteen articles contained in this volume record the contributions and discussions at the 1957 meeting of the Laurentian Hormone Conference. Hormones are chemically diversified and include among their number proteins, polypeptides, steroids, and catechol amines. Their biological effects embrace practically all vital processes, while their applications to medicine include both psychiatry and general medicine. It is therefore inevitable that the good endocrinologist must keep in touch with many different sorts of research, and books like this one assist him to remain in contact with aspects of the subject which otherwise might be difficult to follow.

The first section of the book, devoted to a series of four papers on "Hormone Structure and Function," well illustrates how often small changes in chemical structure can profoundly alter biological activity. The actions considered in this section range from the inhibition or facilitation of the growth of tumours to the regulation of electrolyte metabolism. The next section, dealing with "Hormones in Growth and Development," contains three papers and is largely directed towards hormonal influences on protein and fat synthesis and catabolism. It includes a discussion of the effects of hormones in cattle production, and in mammary development and function. The third section is concerned with "Reproduction", and from this section can be seen how developmental abnormalities can profoundly influence the secretion of hormones and how in their turn the activity of hormones can play a part in sex development. The section on "Hormones and Metabolism" includes a discussion on the effect of hormones on lipoprotein and connective tissue metabolism. Finally, a section on "Neurohumors" includes a discussion of adrenaline, noradrenaline and their striking influence on many different processes.

The book includes discussions on subjects which embrace specialized organic chemistry, tumour growth, cattle raising, sex pathology, atherosclerosis, anxiety neuroses, and senescence. Hormones link the tesserae of this fascinating mosaic and it is perhaps not to be wondered at that the variety of subject and diversity of activity in the field of endocrinology continue to challenge many active minds.

F. G. Young

MICROSCOPY WITH X-RAYS

By Dr. V. E. COSSLETT
Cavendish Laboratory, University of Cambridge

THE idea of making a microscope for X-rays goes back to the earliest days of the subject. In the original announcement of his discovery, Röntgen[1] describes an attempt to focus the new radiation and concludes from its lack of success: "das man mit Linsen die X-strahlen nicht konzentrieren kann, ist nach dem Mitgeteilten selbstverständlich". Although later experiments have shown that, in fact, a focusing effect exists, it is so small that an X-ray microscope on the lines of the usual optical microscope would be impracticably long. However, the advantages of using X-rays are so considerable that many attempts have been made to devise systems which do not include lenses. X-rays penetrate matter relatively easily, they offer the possibility of a higher definition of image than with light, and, more important, they are emitted (and absorbed) in a manner specific to the nature and amount of the elements present, so that analysis on the microscale becomes possible.

Reflexion Focusing

If X-rays are incident perpendicularly on a mirror, they pass straight into it and the amount reflected is negligible; but if the beam just grazes the surface, reflexion is almost 100 per cent efficient. Since all materials have a refractive index slightly less than unity for X-rays, total reflexion occurs for a beam passing from air into any physically denser material such as glass or metal. Hence it is possible to focus X-rays with a curved mirror, so long as they fall on it at glancing incidence. The 'critical angle' is very close to 90°, and so the glancing angle has to be less than 1°, at the wave-lengths so far employed. In consequence, scarcely any focusing takes place at right angles to the plane of incidence, so that the image formed by a concave mirror of spherical curvature is drawn out into a line: the focusing is highly astigmatic. As pointed out by Kirkpatrick and Baez[2], however, a second mirror in a plane at right angles to that of the first will produce a stigmatic image. Focusing systems of this type have been developed by Kirkpatrick and his school at Stanford University[3]. The alignment of the mirrors and the position of the object are somewhat critical at such small angles of incidence, and micrometer adjustments are required. Single-stage microscopes only have so far been built, since a compound microscope would involve undue loss of intensity in the succession of reflexions as well as great difficulty in alignment. The best results, obtained with test grids, approach the performance of a good optical microscope. McGee[4] claims a resolving power of about 0·5 micron, using mirrors of radius 8·5 metres and focal length 6·4 cm. To keep the exposure time within reasonable bounds (10–30 min.), he worked with a direct magnification of seven times only.

The obstacles to further development of the reflexion system are of two types: aberrations of the image, and practical difficulties in making the mirror surfaces. Lack of image brightness, giving long exposures, is also a limitation, since the aperture cannot exceed the grazing angle of total reflexion, that is, about 1°. The aberrations of the focusing system can in principle be overcome by using combinations of several mirrors with surfaces of special form — elliptical, paraboloidal, hyperboloidal — as shown by Wolter[5] and Pattee[6]. The correction obtainable with a pair of crossed ellipsoids followed by a pair of crossed paraboloids should give a resolving power of 500 A.—about twice as good as the ultra-violet microscope and four times better than the limit of the optical microscope.

The preparation of such corrected systems demands great skill and patience in polishing the surface to the required degree of perfection—about ten times better than that called for in the best optical practice. Not only must the surface be true to the particular aspherical form computed, it should also be smooth to almost molecular dimensions. For these reasons, it is difficult to estimate the ultimate performance to be expected from the reflexion X-ray microscope. It seems probable that the same definition as in the best optical microscope will be reached, but less likely that the limit predicted by Pattee is within practical possibility.

Contact Microradiography

The simplest way of obtaining enlarged images is by geometrical projection, dispensing with any form of focusing system, as in the usual method of medical radiography. Specimens partially transparent to the radiation, as most will be, form half-tone images rather than simple silhouettes. The limiting factor to the definition obtainable is set by the penumbra, formed at the edge of the true shadow. The definition can be increased, if microscopical detail is sought, either by reducing the size of the source or by moving the specimen close to the viewing screen (or photographic plate). The former, or projection method, allows high direct magnification, by bringing the specimen close to the source. The latter method yields no initial magnification, all the enlargement being obtained by subsequent viewing of the negative through an optical microscope; it has come to be known as 'contact microradiography'. So long as the X-ray source is far enough away for the penumbra to be of negligible width, the definition in the final image is determined by the size of grain in the developed emulsion and by the resolving power of the optical microscope used to enlarge it. With modern fine-grained emulsions, as prepared for spectrographic purposes, these two factors are of about equal order, namely, about 0·2–0·5 micron, so that the best contact microradiographs have almost as good a definition as the best optical micrographs.

The technique was developed, largely in France, between the two World Wars. Since 1946 it has been brought to a high art by Engström and his co-workers in Stockholm, especially for cytological studies. The first recorded application was to the study of the internal structure of alloys, however. Heycock and

* Substance of a Friday Evening Discourse delivered at the Royal Institution on February 6.

Neville[7], at the laboratory of Sidney Sussex College, Cambridge, in 1898 investigated the differences in size and shape of the precipitates formed when an aluminium–gold alloy was cooled quickly and slowly. Since that time the method has been gradually introduced into metallurgical practice, as in the work of Sharpe[8] and Andrews and Johnson[9] in Great Britain, and Trillat[10] in France. The applications in biology have been much more widespread, and have been recently reviewed by Engström[11]. By using very soft X-rays, it is possible to show clearly even cell components so small as a chromosome, without staining or otherwise interfering with them.

The great advantage of the contact method lies in its simplicity, and in the large field of view recorded. No special X-ray tube is required, though the exposure time can be cut appreciably (to 20 min. or so) by using one properly designed for the purpose[12,13]. The fine-grained emulsions demand careful handling, in dust-free conditions, on account of the high subsequent magnification that is needed. For highest definition, the specimen must be prepared as a thin section and brought into close contact with the emulsion, and Engström has developed special techniques for this purpose. In definition the ultimate limit is set by the resolving power of the optical microscope, so that a resolving power better than about 0·2 micron is not to be expected. The best contact micrographs from Engström's laboratory already approach this limit.

Projection Microradiography

In the projection method the specimen is placed close to the source, instead of close to the photographic plate, and a point source becomes the essential requirement in place of an ultra-fine grained emulsion. A point source is most simply obtained by placing a small aperture before an X-ray source of normal size, and the first 'pinhole-camera' radiographs were made in this way by Czermak in 1896[14]. The intensity of the beam through such an aperture was very small, however, and even with the improved tubes that later became available, it was impossible to combine appreciable magnification with reasonable exposure time (Sievert[15]). In 1939 it was pointed out by von Ardenne[16] that a point X-ray source of great brilliance could be formed by using magnetic lenses to focus the electron beam on to the target of an X-ray tube, the definition in the projection image being then limited primarily by the size of the source.

A point-projection system of this type was later constructed by Cosslett and Nixon[17] at the Cavendish Laboratory. To obtain a high demagnification of the electron source (a tungsten filament), two electron lenses are employed. The electron spot at the target can then be made smaller than 1 micron without difficulty, and even as small as 0·1 micron. The X-rays issue from this focal spot through the very thin target, which serves as window to the tube. An object placed close to it will form an enlarged image on a screen or plate at a distance. Since the source–object distance may be as small as 0·1 mm., a direct X-ray magnification of 100 × is obtainable in a camera-length of 1 cm., or of 1,000 × in 10 cm. The voltage on the tube has usually been in the range 5–20 kV., and the electron beam current is of the order of 1 μamp., concentrated into a focal spot of 1 micron or less. The dissipation of heat from such a small electron spot is so favourable that no water cooling of the target is needed.

The range of applications of the projection method is somewhat wider than that of the contact method, which is restricted by the need to have the specimen close to the recording emulsion. For example, it is better suited to the study of living subjects. Saunders[18] has followed up the earlier contact work of Barclay[19] and Bellman[20] in the field of microarteriography, and has studied the circulation in the ear of the rabbit under the influence of various treatments. The thickness of specimen that can be tolerated is greater than in the contact method, and may be as much as 1 mm. Smith[21], for example, has investigated the musculature of a series of beetles in the projection X-ray microscope. The whole of the internal structure is visible at once, whereas by optical techniques it would have to be worked out from a set of consecutive sections through the insect, owing to the limited depth of focus of the light microscope. A large number of other applications in mineralogy and metallurgy, in entomology and in botany, have also been explored.

In the projection system the depth of focus, in effect, extends all the way from the near side of the object to the photographic plate. It is thus possible to make stereophotographs of specimens much thicker than could be tolerated in the optical microscope; in the latter the focal depth decreases as the useful magnification increases, so that stereophotography at high power is impracticable. Excellent X-ray stereomicrographs have been obtained of alloy structures and of insects. Although it is possible to make stereophotographs by the contact method, it is more trouble both to make the initial pictures and to identify the same area in the two negatives.

The projection method is to be preferred for microradiography whenever the area of interest can be readily identified, by optical or other means, and mounted in the X-ray beam for the production of a direct image. On the other hand, the contact method provides a much larger initial field of view, from which areas can later be selected by exploration of the micronegative under the optical microscope. The exposure-time of the projection method is shorter by a large factor, being of the order of 1 min. The resolving power is limited, not by that of the optical microscope, but by the size of the electron spot on the target and the extent to which electrons are scattered in it before producing X-rays. The best results so far obtained by Nixon[22] and by Ong and Le Poole[23] are in the region of 0·1 micron, the limit of resolution of the ultra-violet microscope. The projection method thus already gives a higher resolving power than either the reflexion or contact methods. Further progress is at present hindered by lack of intensity, since the current that can be focused into the focal spot falls rapidly as its diameter is made smaller, but there is good reason for supposing that the resolving power will be improved to 0·05 micron (500 A.) and fair hope that it will reach 250 A.

Microanalysis by Differential Absorption

Both the contact and the projection methods of microradiography may be used to determine the nature and proportion of the chemical elements in a specimen, by making use of the steps which occur in the absorption spectra. The absorption coefficient of a given element changes abruptly at a well-defined wave-length, whereas that of the matrix in general increases smoothly and approximately in proportion

to the cube of the wave-length. The steps are relatively infrequent, so that it is rare for the wave-lengths for two elements to be too close to be separable with an X-ray spectrometer. If measurements of X-ray transmission are made at wave-lengths just below and just above that of a step, then the difference will be due to the presence of the corresponding element, after allowing for the small change in absorption by the matrix. Knowing the height of the absorption step for the pure element, the observed height gives at once the percentage of it present in the matrix. By this means practically all elements in the Periodic Table may be determined quantitatively.

In the contact technique it is necessary to take micrographs of the specimen at two selected wave-lengths for each of the elements to be estimated. The difference in absorption in particular regions is found by measuring the relative transparency of the negative with a microphotometer. For accurate work, the photographic processing has to be carefully standardized, and it is preferable to make measurements on the original negatives rather than on enlargements. The smallest area that can be analysed is then limited to that of the smallest light spot which can be formed in the microphotometer—about 5 sq. microns. Lindström[24] has carried out micro-analyses of sulphur, phosphorus and calcium in sections of certain biological tissues, with an ultimate sensitivity of between 10^{-11} and 10^{-12} gm. and a relative accuracy of $\pm$ 3 per cent. On the other hand, if only the amount of matter present per unit area is of interest, as in many cytological investigations, an absorption measurement at a single wave-length is sufficient, provided that the composition of the absorber is known within certain limits. By this means Engström[11] has been able to make determinations of the dry weight of cell constituents with an accuracy almost as good as that achieved by the interference microscope.

Absorption microanalysis is more easily carried out by the projection method, since it is then unnecessary to make a photographic negative: the relative transmission of different points in the specimen is measured directly by placing an aperture, behind which is a spectrometer and counter, at the required points in the projected image. Röckert[25], in conjunction with Long[26], measured the micro-distribution of calcium in the cementum layer of the teeth of monkeys of different ages, and taken from different positions in the jaw. It turned out that there was no significant trend: the variation for a tooth of given type, in different individuals, was much greater than the variation in the average value with age or position. Long has used the same technique for determining calcium in a number of minerals, and Mosley and Wyckoff[27] have made qualitative surveys of other elements.

Emission Microanalysis

An alternative method of elementary analysis is by spectrometry of the characteristic emission lines excited when the specimen is made the target in an X-ray tube. From the position of the lines the elements present may be identified and intensity measurements give their relative concentration. By comparison with pure standard samples, the absolute amount of each element can be calculated.

The method has been practised on large-scale specimens for a long time, both by direct excitation on the target of a tube and by fluorescent excitation, in an intense primary X-ray beam. Its application on the micro-scale was first made by Castaing and Guinier[28]. They employed electron lenses to form a point focus of electrons on the specimen, placed inside the tube, and analysed the X-rays excited at a particular point in its surface, selected by means of an optical microscope built into the electron lens. Improvements now allow all elements in the Periodic Table, from uranium down to aluminium, to be analysed to an accuracy of 0·1 per cent in areas of diameter about 1 micron. Such a static spot X-ray microanalyser is now in production in France.

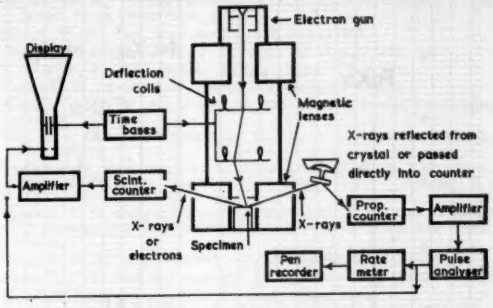


Fig. 1. Schematic diagram of X-ray scanning microanalyser

It would be helpful to be able to see exactly what point in the surface of the specimen was being struck by the electron spot. The flying-spot technique, already applied in optical and electron microscopy, was therefore combined with the microanalyser[29], as shown schematically in Fig. 1. The X-ray output is detected with a counter, amplified and used to modulate the beam of a cathode-ray tube operating in synchronism with the scan of the electron beam across the target. If the undifferentiated output is so displayed, an image is obtained of the specimen surface in terms of its total emissivity of X-rays. If a particular emission line is selected, the image shows the distribution of the corresponding element over the specimen, the local brightness depending on the amount of the element present at that particular point. By 'tuning-in' to each element present, a semi-quantitative analysis of the area scanned can be quickly carried out. When the elements are not near neighbours in the Periodic Table, the necessary selection of the emission lines can be made with a proportional counter alone, but with next neighbours it is at present essential to use a Bragg spectrometer. The collected intensity is then lower and a slow scan has to be used, the image being formed on a screen of appreciable 'memory' (long-delay tube), in order to gain a storage effect.

If a more accurate analysis is required, the electron spot can be made to scan along a given line and the output fed to a pen recorder, which traces out the variation in amount of the selected element across that part of the surface. Alternatively, the scan may be stopped and the electron spot moved by manual control to a point of special interest. The relative intensities of the X-ray lines of the various elements present are then recorded on a chart as the spectrometer selects them from the emitted spectrum (Fig. 2). After correction for differential absorption in the target and recording system, an accuracy of 0·05 per cent can be obtained in favourable cases, but the accuracy falls with the amount of element

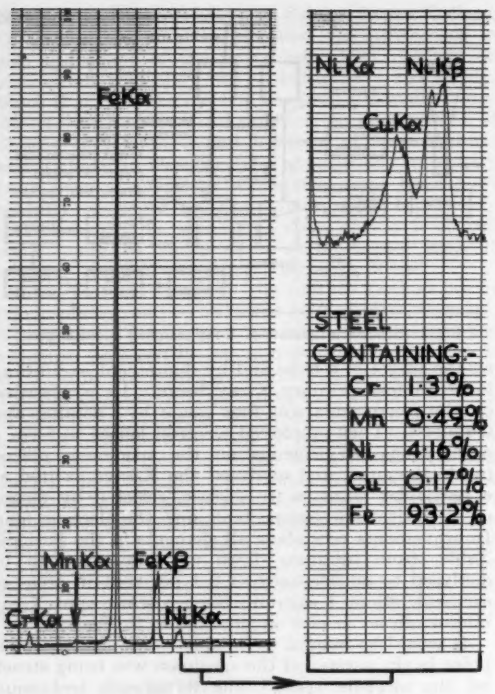


Fig. 2. Analysis of steel with the X-ray microanalyser

present. The electron spot can be made as small as 1 micron in diameter without undue loss in signal strength, so long as a high proportion of an element is present. At the voltages used on the tube (15–25 kV.), the electron beam penetrates a few microns into most metals before its energy falls too low for excitation of their characteristic X-ray lines. A volume of a cubic micron or so can thus be analysed, containing a mass of about 10^{-11} gm., and the sensitivity, the smallest amount that can be detected, is between 10^{-14} and 10^{-15} gm. Although some methods of analysis can give greater accuracy of determination, and one or two methods can equal the sensitivity, none can provide anything like the same degree of microscopic localization as in the X-ray microanalyser. For this reason it is finding increasing application in metallurgical[30], mineralogical[26,31,32] and, to a lesser extent, biological research.

The original scanning microanalyser was made by adapting an old electron microscope. An improved model has now been constructed at the Tube Investments Research Laboratories, Hinxton, which will be used for metallurgical investigations. It allows three spectrometers to be trained on the specimen, so that three elements can be analysed simultaneously. The scanning system has now also been incorporated in the Castaing-type microanalyser constructed at the A.E.I. Research Laboratory, Aldermaston, and shown at this year's Physical Society Exhibition. A simpler instrument has recently been built by Long[26], designed primarily for mineralogical work. Since most rock sections are thin enough to be optically transparent, a microscope is built into the side of the vacuum chamber, so that the section may be searched to select areas of interest for scanning; a fluorescent glow usually appears where the electron spot strikes it. As before, an image is displayed on a long-persistence cathode-ray tube screen, showing the surface distribution of a particular element in the rock, or the variation in concentration along a line may be shown. By using a double-beam display tube, the concentration can be graphed immediately above the trace of the path along which the analysis is made. For high accuracy, a pen recorder may be coupled up in place of the double-beam oscilloscope; but where an analysis to no better than 1 per cent suffices, it may be obtained directly from the photograph of the latter. A number of microanalyses of minerals and meteorites has been made[26,31,32].

The further development of the emission microanalyser faces the difficulty that the X-ray intensity decreases as the size of spot is reduced. Duncumb has analysed precipitates as small as 0·25 micron, with a spot rather larger, but the signal was very weak. Analyses of still smaller regions can certainly be made with the instrument as it is; but the time required becomes prohibitive for routine operation, and the output is insufficient for visual observation. Intensity might be increased in three ways: by improved design of the electron gun; by correction of the spherical aberration of the electron lenses; and by collecting a greater proportion of the emitted X-rays. Research on all three lines is in progress in the Cavendish Laboratory and elsewhere. There is reasonable prospect of substantial improvements, especially in the third direction, and we may expect to be able to analyse particles as small as 0·1 micron in the near future.

The other main problem is the extension of the range of elements that can be analysed, from aluminium down to nitrogen and carbon. The detection and separate estimation of these elements is of great importance for ferrous metallurgy, where carbides and nitrides abound. If this can be done—and the prospects are encouraging[33,34]—then the scope of the X-ray microanalyser will be greatly increased, and it might also become of value to the biologist. At the moment, it is possible to detect and estimate the concentration only of heavier elements that may be present in pathological conditions, such as tin or silica in the lung. Even when carbon, nitrogen and oxygen can be analysed, however, it remains doubtful if it could be done without charring the tissue or otherwise removing constituents selectively by the electron bombardment. The usefulness of emission microanalysis for the biologist must therefore remain an open question until more experience has been gained. In metallurgy and mineralogy its value is already established, and it is now a question of exploring its limits. The peculiar combination of microscopic imaging with microanalysis appears to be more powerful, within its range of resolution, than any other optical or electron optical methods yet devised, so that the new technique, whether or not one admits its classification as a microscope, is rapidly finding a place of its own among research tools.

[1] Röntgen, W. C., *Sitz. Med.-phys. Ges. Würzburg*, 137 (1895).
[2] Kirkpatrick, P., and Baez, A. V., *J. Opt. Soc. Amer.*, **38**, 766 (1948).
[3] Kirkpatrick, P., and Pattee, H. H., "Handbuch der Physik", **30**, 305 (1957).
[4] McGee, J. F., "X-ray Microscopy and Microradiography", 164 (Acad. Press, New York, 1957).
[5] Wolter, H., *Ann. Physik, Lpz.*, **10**, 94, 286 (1952).
[6] Pattee, H. H., "X-ray Microscopy and Microradiography", 135 (Acad. Press, New York, 1957).
[7] Heycock, C. T., and Neville, F. H., *Trans. Chem. Soc. Lond.*, 73, 714 (1898); *Phil. Trans. Roy. Soc.*, **194**, 201 (1900).

[1] Sharpe, R., "X-ray Microscopy and Microradiography", 590 (Acad. Press, New York, 1957).
[2] Andrews, K. W., and Johnson, W., "X-ray Microscopy and Microradiography", 581 (Acad. Press, New York, 1957).
[3] Trillat, J. J., *Rev. Sci.*, Paris, **78**, 212 (1940); *Rev. Métall.*, **46**, 79 (1949).
[4] Engström, A., "Physical Techniques in Biological Research", **3**, chapter 10 (Acad. Press, New York, 1956).
[5] Engström, A., and Lundberg, B., *Exp. Cell. Res.*, **12**, 198 (1957).
[6] Combée, B., and Engström, A., *Biochim. Biophys. Acta*, **14**, 432 (1954).
[7] Czermak, P., *Ann. Physik, Lpz.*, **60**, 760 (1896).
[8] Sievert, R., *Acta Radiol.*, Stockh., **17**, 299 (1936).
[9] Ardenne, M. von., *Naturwiss.*, **27**, 485 (1939).
[10] Cosslett, V. E., and Nixon, W. C., *Nature*, **168**, 24 (1951); *J. App. Phys.*, **24**, 616 (1953).
[11] Saunders, R. L. de C. H., "X-ray Microscopy and Microradiography", 561 (Acad. Press, New York, 1957); *Nature*, **180**, 1353 (1957).
[12] Barclay, A. E., "Micro-arteriography" (Blackwell, Oxford, 1951).
[13] Bellman, S., *Acta Radiol.*, Stockh., Supp. 102 (1953).
[21] Smith, D. S., "X-ray Microscopy and Microradiography", 492 (Acad. Press, New York, 1957).
[22] Nixon, W. C., *Proc. Roy. Soc.*, A, **232**, 475 (1955).
[23] Ong, S. P., and Le Poole, J. B., *App. Sci. Res.*, **7**, 233 (1958).
[24] Lindström, B., *Acta Radiol.*, Stockh., Supp. 125 (1955).
[25] Röckert, H., *Acta Odont., Scand.*, **16**, Supp. 25 (1958).
[26] Long, J. V. P., *J. Sci. Instr.*, **35**, 323 (1958); thesis, Cambridge (1959).
[27] Mosley, V. M., and Wyckoff, R. W. G., *J. Ultrastructure Res.*, **1**, 337 (1958).
[28] Castaing, R., and Guinier, A., Proc. Conf. Electron Microscopy, Delft, 1949, p. 60 (Martin Nijhoff, Delft, 1950). Castaing, R., thesis, Paris (1951).
[29] Cosslett, V. E., and Duncumb, P., *Nature*, **177**, 1172 (1956); "X-ray Microscopy and Microradiography", 374 (Acad. Press, New York, 1957).
[30] Melford, D. A., and Duncumb, P., *Metallurgia*, **57**, 341 (1958).
[31] Long, J. V. P., and McConnell, J. D. C., *Miner. Mag.* (in the press).
[32] Agrell, S. O., and Long, J. V. P. (unpublished work).
[33] Mulvey, T., and Campbell, A. J., *Brit. J. App. Phys.*, **9**, 406 (1958).
[34] Dolby, R. M., *Proc. Phys. Soc.*, **73**, 81 (1959).

HETEROGENEITY IN DEOXYRIBONUCLEIC ACIDS

I. Dependence on Composition of the Configurational Stability of Deoxyribonucleic Acids

By Dr. JULIUS MARMUR and Prof. PAUL DOTY

Department of Chemistry, Harvard University, Cambridge, Mass.

THE base-pair interactions that unite the two chains of deoxyribonucleic acid in its native, helical configuration are of two kinds: adenine–thymine and guanine–cytosine[1]. Because these pairs involve different numbers of hydrogen bonds[2] and are otherwise dissimilar, it is to be expected that they contribute differently to the stability of the helix. We wish to report here the magnitude of this effect (which is substantial), its use in estimating the extent of heterogeneity in composition within samples of deoxyribonucleic acid and to some degree within its molecules, and its importance in inactivation studies.

Upon heating neutral solutions of deoxyribonucleic acid, striking changes in some properties[3] (for example, viscosity, extinction coefficient, light scattering and optical rotation) are known to occur in a relatively narrow range of temperature. These changes reflect a transition from the double-stranded helical configuration to a disordered coil: the transition is essentially the melting of one-dimensional crystallites and may be characterized by the temperature at the midpoint of the transition, T_m. In earlier studies[4] on calf thymus deoxyribonucleic acid there appeared some indications that the transition consisted of a series of overlapping transitions of individual deoxyribonucleic acid molecules having different melting temperatures.

In particular, the transition was broader than expected for such very long, perfectly formed helices, and at temperatures within the transition region the viscosity would quickly reach a constant value and fall further only if the temperature was increased[5]. Furthermore, electron microscopy revealed that within the transition region only two types of deoxyribonucleic acid molecules could be discerned, namely, completely denatured or native ones[6]. The most reasonable explanation lay in assuming that the two hydrogen-

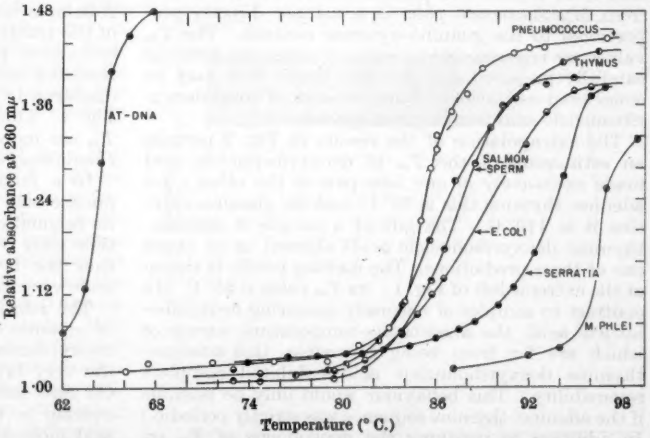


Fig. 1. Variation in absorbance (260 mμ) as a function of the temperature of the deoxyribonucleic acid solution. The result for bacteriophage T_4r deoxyribonucleic acid is not plotted because of serious overlap with *D. pneumoniae*. It was sharper and had T_m equal to 84°. In all cases the solvent was 0·15 *M* sodium chloride plus 0·015 *M* sodium citrate. The samples of bacterial deoxyribonucleic acid were deproteinized by successive mild shakings with chloroform and *iso*amyl alcohol. The samples of animal deoxyribonucleic acid were prepared by the Simmons technique using detergents (see ref. 5). The bacteriophage T_4r deoxyribonucleic acid was a gift from Mr. J. Fleichman

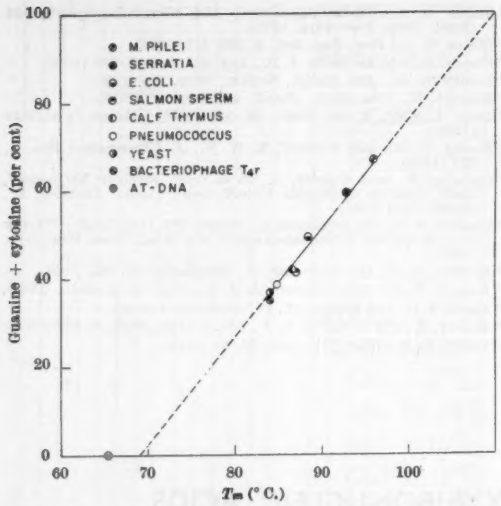


Fig. 2. Dependence of the denaturation temperature, T_m, on the guanine–cytosine content of various samples of deoxyribonucleic acid

bonded base pairs (guanine–cytosine and adenine–thymine) contributed unequally to the stability of the helix, thereby causing T_m to depend on the mean composition of either whole deoxyribonucleic acid molecules or large segments thereof.

In order to test this proposal, we have prepared a number of protein-free samples of deoxyribonucleic acid of different base composition from various sources (see Fig. 1) and determined their transition profiles by measuring the absorbance at 260 mμ as a function of the temperature of the solution. These are recorded in Fig. 1, and the melting temperatures are plotted as a function of the guanine–cytosine content[7] in Fig. 2. In the solvent used (0·15 *M* sodium chloride and 0·015 *M* sodium citrate), it is seen that T_m ranges from 85° C. for deoxyribonucleic acid from *Diplococcus pneumoniae* to 97° C. for that from *Mycobacterium phlei* in a manner directly proportional to the guanine–cytosine content. The T_m values are reproducible to within 0·5 deg. for different carefully prepared samples, and hence they may be considered as intrinsic characteristics of total deoxyribonucleic acid from a given species.

The extrapolation of the results in Fig. 2 permits an estimation of the T_m of deoxyribonucleic acid made exclusively of one base-pair or the other; for adenine–thymine this is 69° C. and for guanine–cytosine it is 110° C. The gift of a sample of adenine–thymine deoxyribonucleic acid[8] allowed us to check one of these predictions. The melting profile is shown at the extreme left of Fig. 1; its T_m value is 65° C. (In contrast to samples of naturally occurring deoxyribonucleic acid, the absorbance–temperature curves of which are far from being reversible, this adenine–thymine deoxyribonucleic acid exhibited complete reversibility. This behaviour would only be possible if the adenine–thymine sequence was strictly periodic.) In addition to verifying the dependence of T_m on composition, the magnitude of the hyperchromicity observed for this sample (52 per cent) supported the trend seen in the naturally occurring deoxyribonucleic acid samples between the hyperchromicity and the composition. In these the hyperchromicity decreased from about 44 to 34 per cent as the guanine–cytosine content increased from 38 to 67 per cent. Thus, in the limit of pure guanine–cytosine deoxyribonucleic acid, the hyperchromicity would probably be only about 25 per cent. Of course, this can only be a rough correlation, because the hyperchromicity in a given sample probably depends on sequence as well as overall composition.

An estimate of the dispersion of the guanine–cytosine content among the molecules of a given sample can be made in the following way. The melting profile of the adenine–thymine deoxyribonucleic acid, since it is of high molecular weight ($S_{20,w} = 12{\cdot}6\ S.$) and with no heterogeneity due to composition, can be assumed to represent the natural transition width due to the intrinsic nature of this co-operative phenomenon. The displacements of this curve from its T_m can then be subtracted from the other melting profiles to give corrected profiles that should reflect only the spread due to heterogeneity of composition. This has been done for the samples studied with the following results. The transition of T_4r bacteriophage deoxyribonucleic acid had the same sharpness as the adenine–thymine deoxyribonucleic acid; hence the deoxyribonucleic acid molecules in this case appear to have essentially identical composition. The profiles for *D. pneumoniae* and *M. phlei* were only slightly broader. The remaining samples of bacterial deoxyribonucleic acid showed increased broadening of the transition, indicating significant heterogeneity in composition. The broadest profile is that from calf thymus; the central part of this transition (50 per cent of the rise in absorbance) covers about 4°. Since the slope of the line in Fig. 2 equates a change of 1 deg. in T_m to a 2·5 per cent change in composition, the half-width of the distribution of guanine–cytosine residues in calf thymus deoxyribonucleic acid can be estimated as extending $\pm$ 5 per cent about the mean of 49 per cent guanine–cytosine.

With calf thymus deoxyribonucleic acid appearing to have a relatively broad distribution of composition, an attempt was made to recover undenatured deoxyribonucleic acid from a partially denatured sample and to see if it had a higher melting profile. A sample heated to 90°, corresponding to 80 per cent of the transition, was cooled and carried through two successive precipitations with streptomycin. The resulting sample exhibited a melting curve that was displaced 1·5 deg. higher than the calf thymus curve in Fig. 1. Thus, it is seen that components of different T_m are indeed contained within calf thymus deoxyribonucleic acid.

In a further experiment, calf thymus deoxyribonucleic acid was degraded sonically to one-twelfth of its original molecular weight. Its melting profile was then only very slightly broadened. This indicated that the heterogeneity observed here exists on a scale very much smaller than that of whole molecules.

The relatively small dispersion in the distribution of guanine–cytosine among the samples of bacterial deoxyribonucleic acid, viewed in conjunction with the very broad range of mean composition, leads to the interesting conclusion that there is essentially no overlap in the composition of the deoxyribonucleic acid molecules of many bacteria. This point is confirmed and discussed in the following communication.

The relation between the transition profile and the inactivation of the transforming ability of *D. pneumoniae* deoxyribonucleic acid has been examined in two ways. When this deoxyribonucleic acid undergoes extensive inactivation by ultra-violet irradiation,

no significant changes in its macromolecular properties can be detected. However, the transition profile is relatively sensitive to irradiation, being shifted to successively lower temperatures. For example, at 10 per cent remaining streptomycin transforming activity, T_m is 1·5 deg. lower. Thus, what is generally considered to be a very small amount of chemical alteration can be detected by the alteration of the absorbance–temperature profile.

When the thermal inactivation of various genetic markers is compared with the transition profile, it is found that the inactivation generally lags behind to varying degrees. This may arise either from a partial reversibility of the transition, that is, a re-healing of some molecules that are only partially melted out, or from our happening to select markers that are carried by the deoxyribonucleic acid molecules having higher T_m values.

We wish to thank Miss D. Lane, Mr. J. Kucera and Mr. L. Matthews for essential contributions to experimental work presented here. We are grateful to Drs. J. Fresco and N. Sueoka for helpful discussions. This investigation has been supported by a grant from the U.S. Public Health Service, C-2170.

[1] Watson, J. D., and Crick, F. H. C., *Proc. Roy. Soc.*, A, **233**, 80 (1954).
[2] Pauling, L., and Corey, R. B., *Arch. Biochem. Biophys.*, **65**, 164 (1956).
[3] See, for example, Doty, P., *J. Cell. Comp. Physiol.*, **49** (1), 27 (1957).
[4] Doty, P., Boedtker, H., Fresco, J. R., Haselkern, R., and Litt, M., *Proc. U.S. Nat. Acad. Sci.*, **45**, 482 (1959).
[5] Rice, S. A., and Doty, P., *J. Amer. Chem. Soc.*, **79**, 3937 (1957).
[6] Hall, C. E., and Litt, M., *J. Biophys. Biochem. Cytology*, **4**, 1 (1958).
[7] Taken from the compilation by Chargaff, E., "The Nucleic Acids", 307 (Academic Press, New York, 1955), and Lee, K. Y., Wahl, R., and Barbu, E., *Ann. Inst. Pasteur*, **91**, 212 (1956).
[8] This sample was a gift from J. Adler, I. R. Lehman and A. Kornberg. See Lehman, I. R., *et al.*, *Proc. U.S. Nat. Acad. Sci.*, **44**, 1191 (1958).

II. Dependence of the Density of Deoxyribonucleic Acids on Guanine–Cytosine Content

By Dr. NOBORU SUEOKA, Dr. JULIUS MARMUR and Prof. PAUL DOTY

Departments of Biology and Chemistry, Harvard University, Cambridge, Mass.

The discovery by Meselson, Stahl and Vinograd[1] of a technique of banding deoxyribonucleic acids in the density gradient established in the cell of an analytical ultracentrifuge has made possible the precise determination of the density of samples of deoxyribonucleic acid in microgram amounts. With this sensitive method available, it was of interest to see if a variation of density with guanine–cytosine content could be detected that would parallel that found for the denaturation temperature, reported in the foregoing communication. A systematic examination of samples of deoxyribonucleic acid having different guanine–cytosine contents showed that there was indeed a significant dependence. We wish to summarize these results here and to indicate the use of this effect in estimating the heterogeneity of composition in deoxyribonucleic acid samples and in studying the denaturation of deoxyribonucleic acid. The estimates of heterogeneity of composition for samples of bacterial deoxyribonucleic acid are unexpectedly small; the implications of this are briefly discussed.

The experimental technique employed was precisely that described by Meselson *et al.*[1]. About 3 μgm. of deoxyribonucleic acid was added to a concentrated solution of cæsium chloride (about 8 *M*) adjusted to the anticipated density. This was centrifuged in the 'Spinco' Model E ultracentrifuge at 44,770 r.p.m. at 25° C. for 24 hr. By this time the deoxyribonucleic acid had banded and its distribution in the band had become essentially constant. The distribution was recorded by photographing the cell using ultra-violet light and making a microdensitometer trace. As an illustration of the effect observed, Fig. 1 shows a photograph of a solution containing nearly equal amounts of four samples of deoxyribonucleic acid and the corresponding trace. The resolution of the four bands is evident. The results of a systematic study are shown in Fig. 2, where the density is plotted against guanine–cytosine content. It is seen that a linear relation is found; it corresponds to a change of 0·00103 in density per 1·0 per cent change in guanine–cytosine content. Since the uncertainty in determining densities in this way is about 0·001, it follows that the guanine–cytosine content of a sample of deoxyribonucleic acid can be determined from its density with a precision of about 1 per cent.

The extrapolation of the results for naturally occurring deoxyribonucleic acid to the extremes of the composition axis indicates that adenine–thymine deoxyribonucleic acid should have a density of 1·662 and guanine–cytosine deoxyribonucleic acid 1·764. The measured density for the former is 1·680. The difference may be attributed to the adoption of a slightly more compact structure when guanine–cytosine pairs need not be accommodated[2].

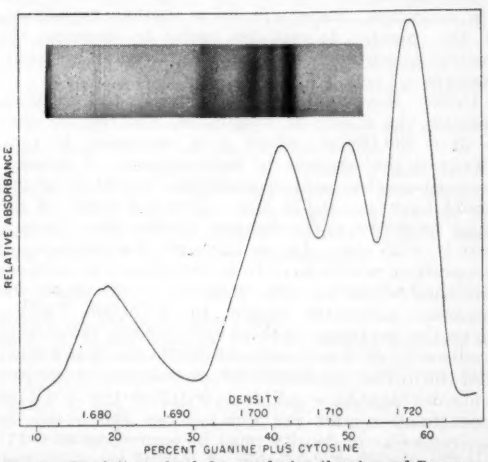


Fig. 1. Resolution of polydeoxy adenine–thymine and *Pneumococcus*, *E. coli* and *Serratia marcescens* deoxyribonucleic acids by density-gradient centrifugation. The photograph was taken after centrifugation at 44,770 r.p.m. for 24 hr. The tracing was taken with a microdensitometer in the region of the four bands. (The composition scale does not apply to the adenine–thymine deoxyribonucleic acid)

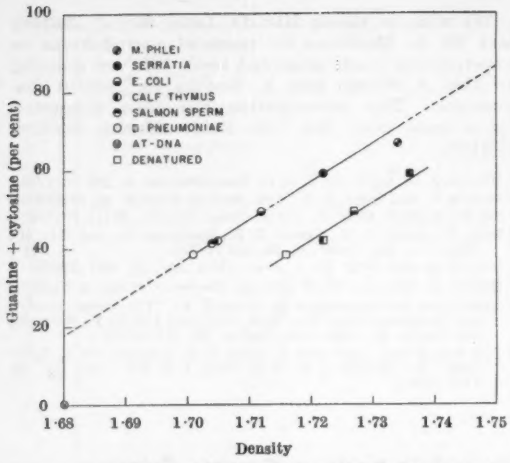


Fig. 2. Relationship of density to the guanine–cytosine content of various samples of deoxyribonucleic acid

At least half the observed dependence of density on composition can be explained by differences in the molar volumes of the two base-pairs as computed from atomic volumes. The failure to explain the entire difference in this way may lie either in errors inherent in deriving molar volumes from atomic volumes or from a small dependence of the atomic co-ordinates of the structure of the deoxyribonucleic acid on base-composition[2], or from an unequal binding of cæsium chloride ions by the two base-pairs.

Having found that the density of deoxyribonucleic acid depends on its composition, it follows that a heterogeneity of composition among the various deoxyribonucleic acid molecules of a given sample will be reflected in a broadening of the band. Molecules of uniform density are distributed in the band region as a result of thermal agitation. Meselson *et al.*[1] have shown that the square of the standard deviation, σ^2, that is, the variance, was inversely proportional to the molecular weight. However, if a distribution of density is superimposed upon the molecules, there will be a further broadening of the band. It will be useful to examine the relative importance of these two effects in a specific example.

Under the conditions employed, the relation between the molecular weight, M, and the variance is $M = 239{,}000/\sigma^2$, where σ is expressed in mm. Thus, in the absence of heterogeneity of density, deoxyribonucleic acid of molecular weight 8,000,000 would have $\sigma = 0{\cdot}172$ mm. The half-width of the band would be approximately double this distance, that is, 0·35 mm. Let us ask what heterogeneity of composition would have to be introduced to increase the band-width by $\sqrt{2}$, that is, to diminish the apparent molecular weight to 4,000,000. Since, under the conditions of these experiments, the density gradient $d\rho/dr$ was equal to $0{\cdot}0122$ mm.$^{-1}$, it follows that the formal dependence of composition on distance is $(d\rho/dr)/(d\rho/dX) = dX/dr = 0{\cdot}0122/0{\cdot}103 = 0{\cdot}1185$ mole fraction unit per mm. Since the σ due to heterogeneity of density must be equivalent to $0{\cdot}172$ mm., a standard deviation of $0{\cdot}0204$ mole fraction units is required in the distribution of composition to provide for an increase in the band width by $\sqrt{2}$. Thus, a distribution of guanine–cytosine such that the half-width of the distribution is 4 per cent on the guanine–cytosine scale would cause a sample of deoxyribonucleic acid having a molecular weight of 8,000,000 to appear to have a molecular weight of only 4,000,000. This degree of heterogeneity of composition lies in the range estimated from the analysis of the absorbance–temperature curves for bacterial deoxyribonucleic acid (see preceding communication). Consequently, the heterogeneity of composition encountered in samples of bacterial deoxyribonucleic acid can be large enough to affect appreciably the molecular weight calculated from band-profiles. Conversely, the band-widths observed for these samples of bacterial deoxyribonucleic acid are sufficiently small to set an upper limit to the heterogeneity in composition. Thus, even if the entire band-profile is interpreted in terms of density heterogeneity, we can conclude that this corresponds to a relatively narrow distribution of molecular composition about the mean composition. Our estimate of the heterogeneity in composition of calf thymus deoxyribonucleic acid is consistent with the fractionations that have been reported: however, only approximate comparisons can be made. The five fractions obtained by G. L. Brown and A. V. Brown[3] indicate a distribution having a little greater dispersion than that which we estimate. The three fractions obtained by Crampton, Lipshitz and Chargaff[4] as well as the selected fractions of Bendich *et al.*[5] show a somewhat smaller range (41–48 per cent guanine + cytosine). Our estimate for the heterogeneity of calf thymus deoxyribonucleic acid is that the σ-value is approximately equal to 0·06 mole fraction units; that is, the half-width of the curve of distribution against composition would extend from about 37 to 49 per cent guanine + cytosine. It is to this point that we shall return at the end.

In the absence of heterogeneity of density, the band-profile should be symmetrical. In samples of two mammalian deoxyribonucleic acids, calf thymus and mouse, pronounced skewness has been found. These are on opposite sides in the two cases. An unsymmetrical heterogeneity of density provides an obvious explanation for this effect.

With the means at hand for estimating the distribution of guanine–cytosine content among the molecules of a sample of deoxyribonucleic acid, it is of interest to see to what extent this heterogeneity is maintained within the deoxyribonucleic acid molecules. Since the distribution of composition is found to be relatively narrow, it would be expected that it would broaden as the molecules were degraded. Since sonic waves can be used to degrade deoxyribonucleic acid without denaturation[6], this problem may be approached as follows. We assume that the total variance of a band is equal to the sum of the variance due to thermal agitation and to the variance due to the distribution of density. If the molecular weight can be obtained for the original deoxyribonucleic acid and for one or more sonically degraded samples, the variance related to molecular weight can be computed. When this is subtracted from that found from the band-profile, the remainder should in each case be that due to the heterogeneity of density, and at sufficiently advanced degrees of degradation it should increase. In a preliminary experiment along these lines we degraded sonically calf thymus deoxyribonucleic acid from its original value of 8,000,000 to 620,000. The broadening of the band-profile was found to be actually somewhat less than that expected from the fall in molecular weight alone and, incidentally, the skewness remained.

Consequently, in this case we conclude that the distribution of composition found for the deoxyribonucleic acid molecules is preserved down to at least a tenth the original molecule.

When deoxyribonucleic acid is thermally denatured, the density increases. The results for four samples are plotted as square symbols in Fig. 2. The average increase of 0·015 compares very well with the earlier observation of an increase of 0·014 for two samples by Meselson and Stahl[7].

In an earlier study of thermal denaturation of calf thymus deoxyribonucleic acid[8], it appeared that the sample consisted of molecules having a range of individual denaturation temperatures, and this can now be interpreted as resulting from the distribution of guanine–cytosine. When partially denatured calf thymus deoxyribonucleic acid was examined in the density gradient, it was found that the band corresponding to the original deoxyribonucleic acid had diminished principally at the expense of material of low density, and that the new band which appeared had a lower density than completely denatured deoxyribonucleic acid. This behaviour clearly indicates that those molecules of relatively low guanine–cytosine content had become preferentially denatured. It has already been mentioned that the isolation of native deoxyribonucleic acid from such a partially denatured sample had a higher melting temperature (see preceding communication). When this fraction was examined in the density gradient, it was found to have a correspondingly higher density than the original deoxyribonucleic acid from which it had been isolated. Thus, the earlier views on the denaturation of deoxyribonucleic acid are confirmed and extended by recognizing explicitly the role of the guanine–cytosine heterogeneity.

In conclusion, we return to comment on the surprising finding that samples of bacterial deoxyribonucleic acid have a relatively narrow range of base composition, while the mean compositions show a wide variation. This stands in contrast to the nearly identical base compositions of mammalian deoxyribonucleic acid. The result for bacterial deoxyribonucleic acid is surprising, because it shows that bacteria having deoxyribonucleic acid density differences of 0·02 or more have essentially no deoxyribonucleic molecules with common composition. Since the metabolism and replication of bacteria do have much in common, it is generally thought that many of their proteins should be identical, or very similar. Yet all current discussions of the way in which deoxyribonucleic acid can control the sequence of amino-acids in protein requires a direct correlation between the composition of the deoxyribonucleic acid and of the protein.

Since it is often assumed that the information in deoxyribonucleic acid passes to the protein through ribonucleic acid, it is of interest to note that Belozersky and Spirin[9] have demonstrated that the variation in composition of bacterial ribonucleic acid is relatively small, but nevertheless it does show a significant correlation with the composition of the corresponding deoxyribonucleic acid for nineteen bacterial species.

It appears that the explanation of this dilemma with regard to bacterial deoxyribonucleic acid must lie with one of three possibilities.

(1) The proteins that perform common functions in bacteria may be structurally quite different in many cases. For example, in enzymes the active sites may be similar, but the main part of the proteins different. This is the situation that might be expected if the evolutionary development of bacteria has followed a number of independent paths, each corresponding to the elaboration of a complex genome from a primary deoxyribonucleic acid of a particular composition. In general, this view suggests that the distribution in composition of deoxyribonucleic acid molecules of organisms reflects their phylogenetic relationship.

(2) The proteins that perform common functions in bacteria are very similar structurally. If all the deoxyribonucleic molecules are genetically active and yet unrelated in composition in many cases, the coding between the deoxyribonucleic acid and the protein must be very different from those that have been considered hitherto[10]; that is, it has been customary to assume that the sequence of amino-acids (20) in proteins is coded in the sequence of the four nucleotides in deoxyribonucleic acid. Since it has been shown that various triplets of nucleotides are sufficient to specify each amino-acid, it follows that similar proteins must be derived from deoxyribonucleic acid molecules of similar composition, unless quite artificial, *ad hoc*, restrictions are imposed. If the coding concept is to remain, a radical revision is necessary in order to eliminate this dependence on composition. For example, the four-letter alphabet (adenine, cytosine, guanine, and thymine) could be abandoned in favour of a two-letter alphabet in which adenine and cytosine (6-amino-bearing nucleotides) would serve as one letter and guanine and thymine (6-keto-bearing nucleotides) would serve as the other. In this case no relation to composition would be expected. Of course, it would be possible to retain the four-letter alphabet if it were assumed that different codes applied to different bacteria, but this alternative is unattractive because it is most unlikely that different codes would yield the same number of amino-acids (20).

(3) The proteins that perform common functions in bacteria are very similar structurally, and the current ideas concerning the coding problem remain acceptable. This could only be true if the major part of the deoxyribonucleic acid was genetically inactive. The minor part of the deoxyribonucleic acid could then have a composition in common with the corresponding part of other bacterial deoxyribonucleic acid. It appears that this minor part would have to be less than 10 per cent of the whole to have escaped detection.

The proof, or even the disproof, of any one of these possibilities would be very enlightening.

We wish to thank Prof. M. Meselson and Mr. J. Kucera for help in the initial stages of this investigation. This work has been supported by grants from the U.S. Public Health Service, *C*-2170, and from the National Science Foundation.

[1] Meselson, M., Stahl, F. W., and Vinograd, J., *Proc. U.S. Nat. Acad. Sci.*, **43**, 581 (1957).
[2] Spencer, M., *Acta Cryst.*, **12**, 59 (1959).
[3] Brown, G. L., and Brown, A. V., "The Biological Replication of Macromolecules", 6 (Academic Press, New York, 1958).
[4] Crampton, C. F., Lipshitz, R., and Chargaff, E., *J. Biol. Chem.*, **211**, 125 (1954).
[5] Bendich, A., *et al.*, *J. Amer. Chem. Soc.*, **80**, 3949 (1958).
[6] Doty, P., McGill, B., and Rice, S. A., *Proc. U.S. Nat. Acad. Sci.*, **44**, 424 (1958).
[7] Meselson, M., and Stahl, F. W., *Proc. U.S. Nat. Acad. Sci.*, **44**, 671 (1958).
[8] Doty, P., Boedtker, H., Fresco, J. R., Haselkorn, R., and Litt, M., *Proc. U.S. Nat. Acad. Sci.*, **45**, 482 (1959).
[9] Belozersky, A. N., and Spirin, A. S., *Nature*, **182**, 111 (1958).
[10] Crick, F. H. C., in "The Biological Replication of Macromolecules", 138 (Academic Press, New York, 1958).

NEWS and VIEWS

Geology at Exeter: Dr. Scott Simpson

Dr. Scott Simpson, at present lecturer in the University of Bristol, has been appointed professor of geology in the University of Exeter in succession to Prof. A. Stuart, who has retired (see *Nature*, 181, 87; 1958). After receiving his early education at Highgate School, Scott Simpson proceeded to Clare College, Cambridge, and in 1937 took the second part of the Tripos in geology, selecting the alternative papers in palæontology. His postgraduate training was at Frankfurt-am-Main, where the late Prof. Rudolf Richter set him the task of unravelling the structurally complex area of Devonian rocks between Mayen and Daun in the east Eifel. That success attended his efforts is clearly shown by the fact that Richter, rather characteristically, arranged for Scott Simpson to be formally awarded his doctorate by the University of Frankfurt-am-Main two years after the start of hostilities between Britain and Germany.

Scott Simpson was assistant lecturer in the Department of geology and mineralogy at the University of Aberdeen for the first two years of the Second World War before he was seconded for the next four years to the Department of Natural Philosophy, where he instructed Royal Artillery and R.A.F. cadets in elementary physics and also worked at the Radon Centre. He transferred in 1949 from a lectureship in geology at the University of Aberdeen to a corresponding academic post at the University of Bristol. Here during the past decade he has acquired a reputation as a recognized authority in Britain on the stratigraphy and palæontology of the Devonian rocks. His own researches in Devon, and those of his students, have already resulted in important correlations being made with Devonian rocks of parts of Germany, and the methods he has applied have resolved the complicated geology of large areas of Devon and Cornwall. Of recent years he has carried out pioneer work in the study of structures in sedimentary rocks due to burrowing organisms, and their interpretation in terms of the interplay of organic and sedimentary processes. His reputation extends beyond the British Isles and his researches are recognized in Germany, Holland and France.

The Editors regret that, due to difficulties in the printing industry, some sections of this issue of "Nature" have had to be curtailed

Development and Research Appointments at the Mond Nickel Co., Ltd.: Dr. W. Steven

Dr. W. Steven, superintendent of the Development and Research Department Laboratory of the Mond Nickel Co., Ltd., in Birmingham, is to be transferred on June 1 to the Development and Research Division of the International Nickel Co., Inc., New York, as director of research. He has also been elected an assistant vice-president of that company. Dr. Steven graduated in the University of Glasgow in 1939 and obtained his Ph.D. degree in 1942. After five years with William Jessop and Sons, Ltd., Sheffield, he joined the Mond Nickel Co. in 1947 as a research metallurgist. In 1955 he was appointed superintendent of the laboratory.

Dr. G. L. J. Bailey

Dr. G. L. J. Bailey has been appointed superintendent of the Mond Nickel Development and Research Department Laboratory in Birmingham in succession to Dr. Steven. He took a B.Sc. (Hons.) degree at the Imperial College of Science and Technology, London, in 1937 and obtained his Ph.D. in 1939. After working at the Admiralty Engineering Laboratory, West Drayton, he joined the British Non-Ferrous Metals Research Association, where he became deputy research manager. Since 1955 he has been superintendent of the Platinum Metals Research Laboratory of the Mond Nickel Development and Research Department at Acton.

Dr. W. Betteridge

Dr. W. Betteridge has been appointed superintendent of the Platinum Metals Research Laboratory of the Mond Nickel Development and Research Department at Acton in succession to Dr. Bailey. Dr. Betteridge graduated in physics at the University of Birmingham in 1931, obtained his Ph.D. in 1933 and his D.Sc. in 1958. After serving with Joseph Lucas, Ltd., and the Bristol Aeroplane Company, he joined the Mond Nickel Co. in 1947, where he has been concerned primarily with the development of high-temperature materials, at first in the Birmingham Research Laboratory and more recently as development officer in London.

Submarine Defence Research

In reply to a question in the House of Commons on April 29, the Parliamentary and Financial Secretary to the Admiralty, Mr. C. Ian Orr-Ewing, said that the North Atlantic Treaty Organization research centre on defence against submarines, which was being set up by SACLANT at La Spezia, would be financed, at least for the initial period, entirely by the United States, and was intended to augment the scientific effort devoted by the several Nato countries to anti-submarine warfare research. The centre would be concerned primarily with basic problems, but would also undertake some operational research. The number of British scientists and Royal Naval officers to be employed there was not yet settled, but the naval adviser on the Scientific Advisory Council which would direct the work of this unit would be the Chief of the Royal Naval Scientific Service.

Department of Scientific and Industrial Research: Lending Library

A list showing holdings of the Lending Library Unit of the Department of Scientific and Industrial Research of irregular serials being received from the U.S.S.R. and Bulgaria has been issued (pp. ii+90.

London: D.S.I.R., Lending Library Unit, 1959), the titles being arranged mainly in alphabetical order, using the Cyrillic alphabet. There is also a short list of serials received from Latvia, Lithuania and Estonia, published in the Roman alphabet. Besides an index of transliterated abbreviations of titles, arranged alphabetically, there is an alphabetical subject-list based on a broad framework of the Universal Decimal Classification. The items listed can be obtained by approved borrowers from the Science Museum Library, using loan requisition forms issued by that Library, but these should be sent direct to the Unit at 20 Chester Terrace, London, N.W.1.

The Royal Institution Library

THE Library of the Royal Institution, which contains about 65,000 volumes, is divided into five sections, comprising the old library—started in 1799 and including many of the early works of the great philosophers as well as books published up to 1857 and the notebooks, letters and papers of Count Rumford, Humphry Davy, Michael Faraday, John Tyndall and others; the modern library—containing books published after 1857, arranged by the Gliss classification; the lending library, devoted mainly to general and popular science; the periodicals, of which 400 are currently received; and the storerooms. Notes on the library and its catalogues, with a list showing the holdings of periodicals and serial publications currently received, are given in a pamphlet recently issued (pp. 28. London: Royal Institution, 1958).

Florida State Museum

THE Florida State Museum serves as a joint University–State Museum and its major functions are to assemble and maintain collections of cultural and natural objects; to encourage research on this material; and to disseminate knowledge by publications and display. The annual report for 1957–58 (pp. 20. Gainesville, Fla.: Florida State Museum, The University, 1959) indicates that these functions have been fully maintained. During the period under review the staff has concentrated on the improvement of display, and many new ideas and techniques, especially those including the effect of artistry in presentation, have been incorporated. The investigation of archæological sites that might have been damaged by road construction has continued.

Tetrahedron Letters

As a supplement to the journal of organic chemistry, *Tetrahedron*, the Pergamon Press (London and New York) has begun to issue fortnightly or monthly *Tetrahedron Letters*, in which short communications of interest to organic chemists will be published within a week or month of acceptance (subscription, post paid (air mail charges extra): £7 or 20 dollars per annum for libraries, government departments and industrial establishments; no extra charge for private subscribers to *Tetrahedron* who write direct to the publisher certifying that the subscription is for their personal use). The first number (pp. 35) was published in March and contains six short communications of an international character. This publication should relieve some of the pressure on other scientific journals and its high standard seems likely to ensure it the success it deserves.

Education of Instrument Technologists and Control Engineers

THE subject of Mr. J. F. Coales's presidential address on May 6 to the Society of Instrument Technology was well chosen. Having shown the growing need for larger numbers of control engineers and instrument technologists and having proved that in most industries to-day there is some form of automatic control, he proceeded to outline the types of courses suitable for the men in charge. His views on the education of control engineers coincide with those expressed by several speakers at the conference on Degree and Diploma Courses in Applied Physics, arranged by the Society of Instrument Technology in November 1956, particularly in his insistence upon a broad-based fundamental approach for the graduate. Appendixes to his address contained valuable information which could not be easily assimilated into the body of the address, and many will be particularly interested in Appendix 2, concerned with detecting elements, and Appendix 3, outlining syllabuses for the ordinary National Certificate in Instrument Technology. Appendix 2 contains some twenty-one examples out of more than two hundred listed in a recent report on "Detecting Elements" published by the British Scientific Instrument Research Association.

Cyanamid International: Technical Papers

CERTAIN technical papers prepared by the research workers of Cyanamid for presentation before various learned societies in the United States or for publication in the proceedings of technical institutions in that country are to be made available in Britain. These papers cover a wide range of subjects but fall roughly into three classifications: pharmaceutical, agricultural and industrial, these being the main spheres of activity in which the Cyanamid group of companies is engaged. From time to time lists are prepared showing details of the technical papers available for distribution. Readers who would like to receive these lists as and when they are issued should write to: Cyanamid International Information Service, 16 Bolton Street, London, W.1.

The Age of the Earliest Remains of Man

CERTAIN skeletal finds from caves in the dolomite formation of the Transvaal not far from Pretoria have been known for some time. Whether these are indeed the earliest known remains of true man or whether they must be considered as still prehuman remains a matter of controversy. The late Dr. van Riet Lowe described in *Nature* (**175**, 16; 1955) a series of chipped stones which he considered to be artefacts, so that the beings in question were tool-using creatures. But grave doubts have been cast on Lowe's opinion and the objects in question may be natural products. Their association with the skeletal remains is more than uncertain, and they are probably later in age. Nevertheless, these skeletal finds are of capital importance, particularly to the physical anthropologist, and it is especially important that the cave deposits containing the remains of the Australopithecinæ should be accurately dated. A detailed investigation by C. K. Brain has recently been published (The Transvaal Ape-Man - bearing Cave Deposits. By C. K. Brain, with an appendix by R. F. Ewer. Transvaal Museum Memoir No. 11. Pp. viii+131. Pretoria: Transvaal Museum, 1958). The book can be recommended as a clear, succinct

and factual account of the problems involved. The first part comprises an account of dolomite caves in general and of their infilling. This part will perhaps be of greater interest to the geologist, but the prehistorian is unwise if he puts these matters completely on one side. Part 2 gives a detailed account of most of the known sites—Sterkfontein, Swartkraus, Kromdraai, Coopers *B*, and the Makapan limeworks. In a final chapter a correlation of the deposits in the various sites is given and their ages suggested. Using the Leakey fourfold system of pluviations, it would seem that these early beings must be assigned to a period between the Kageran and the Kamasian pluvials, that is to say, to the first inter-pluvial, except for Kromdraai, which is slightly younger and dates to the second pluvial period. If they are certainly human and an association with true artefacts can be proved, they are indeed the earliest remains of our species so far discovered. The appendix by R. F. Ewer lists the faunal remains found.

Bibliography on the Chemistry of Tobacco Smoke

"The Constituents of Tobacco Smoke: an Annotated Bibliography", edited by H. R. Bentley and E. G. N. Berry, is the third in a series of research papers published by the Tobacco Manufacturers' Standing Committee (pp. ii+49. London, 1959). This publication, with its attractive format, contains more than 600 references on tobacco smoke, drawn from the technical literature of the world on that subject. The earliest reference quoted is to a German work of the year 1826. There are eight sections, dealing with nicotine, bases, acids, phenols, hydrocarbons, neutral fraction, arsenic, and a miscellaneous section which includes such diverse components as trace minerals, carbon monoxide and free radicals. Each section is preceded by a short introductory comment. While nearly 200 substances are quoted as occurring in tobacco smoke, the editors wisely point out that the degree of precision with which identifications have been made is by no means uniform, so that some of the work reported is of questionable reliability. This publication should prove very valuable to workers in the field of tobacco carcinogenesis, where an active search for carcinogenic factors of human significance is under way, proceeding from the proposed causal hypothesis relating tobacco usage and human cancer. The minute quantities of traditional carcinogens of the aromatic hydrocarbon class found in cigarette smoke are unlikely to explain the proposed special carcinogenic environment of the cigarette smoker.

Books required for the "Oxford English Dictionary"

The Editor of the Supplement to the "Oxford English Dictionary" writes: "It has proved impossible to obtain copies of some works, now out of print, which would help in the preparation of the forthcoming Supplement to the 'Oxford English Dictionary'. Any readers who would be willing to give, sell or lend copies of any of the following are requested to get in touch with the Editor at: 40 Walton Crescent, Oxford.

"J. R. Carpenter, 'Ecological Glossary', 1938.
R. T. Glazebrook, 'Dictionary of Applied Physics', 1922–23.
Oliver Heaviside, 'Electrical Papers', 1892.
Henderson and Henderson, 'Dictionary of Scientific Terms' (we have edit. 6, 1957; earlier editions required).
B. D. Jackson, 'Glossary of Botanic Terms' (any edition).
'Radio Engineering Handbook' (edits. 1 and 2), 1933–35.
A. L. M. Sowerby, 'Dictionary of Photography' (we have the 18th edit., 1957 impression; earlier editions needed)."

Announcements

The Wenner-Gren Foundation for Anthropological Research has awarded Viking Fund Medals and Awards of 1,000 dollars to Prof. Raymond W. Firth (University of London); Prof. Henri V. Vallois (director of the Musée de l'Homme, Paris); and Dr. Jesse D. Jennings (University of Utah).

It was announced at a recent meeting of the British Section of the Louis Rapkine Association that the Cross of Chevalier de la Légion d'Honneur has been awarded by the French Government to Dr. G. E. W. Wolstenholme, director of the Ciba Foundation, in recognition of the work he is doing for international co-operation in medicine and science and more particularly for Anglo-French collaboration.

The Geological Society of London has awarded the Daniel Pidgeon Fund for 1959 in moieties to Dr. M. K. Howarth, who proposes to study the Alum Shales (Upper Lias) at Whitby with particular reference to the ammonite fauna, and to Dr. M. J. S. Rudwick, who proposes to undertake research into the functional significance of the morphology of fossil brachiopods.

Two open days will be held by the British Cast Iron Research Association during May 28 and 29. A number of special exhibits and demonstrations have been arranged. The first day is set aside for representatives of member-firms and for the Press, the second for representatives of Government departments, other research organizations, educational bodies, trade unions, users of iron castings, and others. Further information can be obtained from the Intelligence Department, B.C.I.R.A., Bordesley Hall, Alvechurch, Birmingham.

The fifth International Salon de la Chimie is to be held in Paris during June 18–29 in conjunction with the fifth Rubber and Plastics Exhibition, the eleventh Exhibition of Laboratory Equipment and Instruments, the fifth Exhibition of Chemical Engineering and Protection against Corrosion, the fifth Industrial Heating Exhibition, and the first Literature and Press Exhibition. Further details can be obtained from the General Secretariat of the Fifth Salon de la Chimie, 28 rue St. Dominique, Paris 7e.

The twelfth annual conference on Electrical Techniques in Medicine and Biology will take place in the Sheraton Hotel, Philadelphia, Pennsylvania, during November 10–12. It is sponsored by the Joint Executive Committee in Medicine and Biology, appointed by and representing the pertinent interests of the American Institute of Electrical Engineers, the Institute of Radio Engineers for its group on Medical Electronics and the Instrument Society of America. Proposals for papers are invited and should be submitted to Dr. L. E. Flory, RCA Laboratories, Princeton, N.J., before July 1. Such proposals should be 100–200 words in length, supplemented by a short biographical note, and include affiliation and company title.

Erratum. J. R. Hensler, Bausch and Lomb Optical Co., Rochester 2, New York, writes: "In my note, 'Synthesis of Colour Centres in Silica and their Thermoluminescence', published in *Nature*, March 7, p. 673, column 1, lines 3–6 should read: 'The quenching of the luminescence in the presence of the aluminium centres is evidence for the interaction of these and the ultra-violet absorbing centres'. The phrase 'in the presence' was omitted".

JOACHIM JUNGE (1587–1657)

A FORGOTTEN GENIUS

THE name of Junge, known also as Jung or Jungius, is to be found in few biographical dictionaries, and is seldom mentioned by historians of science. Nevertheless, his contribution to scientific thought and knowledge was significant and considerable. He lived at a time when alchemy became chemistry, astrology gave way to astronomy, and other sciences assumed a new appearance as the old scholastic approach to learning was replaced by a return to the use of experimental methods. Junge published little or nothing during his life-time, and for that reason his work has been overshadowed by that of his contemporaries. To-day, after a lapse of three centuries, his achievement is beginning to receive a well-merited recognition.

The main events of his career may be summarized as follows. Born at Lübeck in 1587, the son of a schoolmaster who died while his son was still a child, he was enabled, by the sacrifice and wisdom of a far-seeing mother, to enrol as a student at Rostock in 1606, and then at Giessen, where his brilliant intellect became so obvious that he was offered, and accepted, the chair of mathematics. This academic position did not afford him the scope for wider study which he desired, and in 1614 he resigned, and went to study medicine at Padua, where he graduated M.D. in 1618. On his return to Rostock he hoped to found an academy of sciences, and although he did succeed in establishing the Societas Ereunetica in 1622, one of the earliest societies of its kind, it survived for only two years. The reason of this failure was that Junge was suspected, though quite unjustly, of being a Rosicrucian, a member of the secret society of that time devoted to astrology. It was not surprising, however, that the progressive views of Junge, which included his advocacy of teaching in the vernacular, and of a greater use of experiment, brought him into disrepute with the Church. As a result of this misfortune, and the threat of an accusation of heresy, he removed to Hamburg, where he passed the last thirty years of his life as rector of the High School, and where he died on September 23, 1657.

Junge's opinions won the approval of Leibniz, who placed him on a level with Galileo, Kepler and Descartes, while Goethe said that if the methods of Junge had been followed sooner, the advance of human knowledge might have been hastened by a century. Unappreciated in his life-time, his service to science remained obscure. Like his contemporary, William Harvey, he lost many of his manuscripts in a fire, and the suspicion with which he was surrounded prevented the publication of those that remained. Unlike Harvey, Junge did not live to see the result of his labours. His disciples and followers recognized their merit, however, and among his works, printed during the century following his death, are "De plantis Doxoscopiae physicae minores" (1662), "Isagoge phytoscopia" (1678), "Logica Hamburgensis" (1681), "Geometrica empurica" (1688), and "Opuscula physica botanica" (1747).

Most of those books have become very rare, but during the succeeding years his memory has been kept alive by his admirers, and especially by those in Hamburg. On the 300th birthday of Junge in 1887, Dr. Konrad Friedlander reported that "in terms of the will of Joachim Jungius, about 500 scholarships had been awarded, for the furtherance of scientific work, and that the list of scholars included many distinguished persons". In 1928, Prof. Meyer-Abich, of the University of Hamburg, founded the "Jungius Kommission", with the view of investigating the unpublished manuscripts, and in the following year a commemorative volume was printed, entitled "Beitrage zur Jungius-Forschung". Eventually, in 1947, a "Joachim Jungius Gesellschaft" was inaugurated in Hamburg, with the wider object of "promoting research in all branches of science, and extending the scope of scientific work".

The year 1957, the tercentenary of his death, marked a further revival of interest. Dr. J. H. S. Green paid a graceful tribute to the memory of Junge in an article in *Nature* (**180**, 570; 1957), and when the Joachim Jungius Gesellschaft met at Hamburg on October 31, 1957, under the presidency of Prof. Kurt Heyns, his first duty was to award a Joachim Jungius Prize and Medal to its first recipient, Prof. Rudolf Meyer, of Zurich, who has long been an ardent student of the works of Junge, and had recently edited a new edition of "Logica Hamburgensis". Prof. Meyer than delivered an address on "Joachim Jungius and the Philosophy of his Time", which, following the introductory remarks of the president, is printed, with other noteworthy papers by various authorities, in the little volume commemorating the occasion, which bears the title "Die Entfaltung der Wissenschaft" (The Development of Science)*, and which may be read with pleasure and profit by every scientist, whatever be his special branch.

Prof. Kurt Vogel deals with "Mathematics in the Early Years of the Seventeenth Century". Prof. R. Hooykaas, of Amsterdam, in a paper on the knowledge of elements and atoms at that time, shows how Junge championed the atomic theory, and opposed the older philosophy, alleging that in spite of chemical changes, atoms retained their individuality. This paper concludes with the significant remark that "one free man can do more for science than a whole crowd of *Konformisten*". The important place of Junge in the history of botany is clearly revealed by Prof. Walther Mevius, who tells us that Junge's contribution to that science was probably his greatest achievement. He was indeed one of the founders of scientific botany, and to him we owe the terminology which was perfected later by Ray and Linnæus. Junge was the first to classify plants according to the varieties of their component parts. Many modern terms are of his coinage, and, as Charles Singer stated in his "Short

* Die Entfaltung der Wissenschaft: Zum Gedenken an Joachim Jungius (1587–1657). (Vorträge gehalten auf der Tagung der Joachim Jungius-Gesellschaft der Wissenschaften, Hamburg, am 31 Oktober/1 November 1957 aus Anlass der 300 Wiederkehr des Todestages von Joachim Jungius.) Pp. 160. (Glückstadt: Kommissions-Verlag J. J. Augustin, 1958.)

History of Biology", Junge "had a real insight for classification, grouping plants according to the formation of their flowers, and naming the groups Compositae, Labiatae and Leguminosae", names which still remain. The paper which follows, on "Experiment in the Medicine of the Seventeenth Century", gives an interesting account of this important epoch in medical history, and is contributed by Prof. J. Steudel, of Bonn. It is illustrated by eleven figures, showing some experiments of Harvey, de Graaf, Boyle, Mayow and others. Prof. W. Flitner, of Hamburg, describes the logic and philosophy of the period, and his colleague, Prof. D. K. Deitrich Schmidt, discusses the changing outlook of theology in the days of Junge.

The scope of this excellent collection of monographs is extended even more widely by a thoughtful and suggestive study by Prof. Karl Bauch, Freiburg, of the new approach to art, adopted by Raphael, Rubens and other artists in the opening years of the century under review. Twelve illustrations in this paper are of much interest. Finally, an appropriate background to the whole scene is provided by Prof. Otto Brunner in an essay on "Hamburg's Historical Position during the Lifetime of Joachim Jungius". This article, also appropriately illustrated, should prove valuable as a reference to the contemporary topography of the city. Too often, in writing of the history of science, this essential basis of social history is omitted. The Joachim Jungius Society is to be warmly congratulated on the publication of this timely and attractive little volume, which not only pays a fitting tribute to the work of a forgotten genius but also builds upon that work an edifice well worthy of so sound a foundation.

Douglas Guthrie

EDUCATION IN THE AGE OF SCIENCE

In a recent issue of *Daedalus* (88, No. 1; 1959), ten distinguished American correspondents examine different aspects of American education and their place in a rapidly evolving scientific and technological age. The articles are grouped in five pairs, each pair representing two different points of view regarding the same aspect of education. The problem of Prof. Sidney Hook and George N. Shuster is that of the general nature and ends of education. Prof. Hook holds that the ends are threefold: to produce skills, knowledge and loyalties. On all three aspects there is a difference of opinion. If 'skills' mean the ability to think, speak and write effectively, education must clearly aim at such skills; but do they include skills of everyday living? Here there are rigorists and latitudinarians. Regarding knowledge and loyalties, the differences go deeper. Prof. Hook is a secularist who believes that the only knowledge worthy of the name is that which is accredited by scientific method, and that even moral judgments are subject to empirical tests. Shuster believes that theology is the queen of the sciences, and that the most sure and important knowledge has another kind of certification altogether.

In the papers of the second group, Prof. Douglas Bush states the case for the humanities in education, and Prof. Ernest Nagel the case for the sciences. Both advocates recognize that both disciplines are essential but they differ about two points. Prof. Bush, concerned about the 'sophisticated vulgarity' of newspapers, best-sellers, radio, advertising and music, thinks that much of this is a by-product of science, partly through its technical applications, partly through the exaltation of science in the popular mind. Science has made 'mass civilization' possible, and 'the religion of commonness' that has resulted has been creeping out from news-stands and films into the schools and universities. Science provides the means by which a commercial civilization may exploit the mind more expertly, but no corresponding resistances against the shoddiness of mass values; nor does it do anything to prevent the 'spiritual impoverishment of the expert' himself.

Prof. Nagel, admitting the part of science in helping to produce mass culture, replies that it is unjust to make science responsible for men's misuses of it, and argues that our mass culture, for all its defects, represents a high-water mark in democratic education. He then makes a claim for science that provides a second bone of contention, believing that scientific method, taken broadly as the method by which propositions are validated, is the sole and sufficient means of establishing any kind of knowledge. The judgments of value passed by a humanist upon poetry or music may be merely expressions of his feeling; but if they say more than that, if they offer themselves as true, there is only one way of establishing them, and that is the approved way of science. Bush, Shuster and others do not agree. They hold that the poet, the religious man, and the critic have insights that such a method is inadequate to appraise. Here, as elsewhere, it becomes clear how difficult it is to talk about education without entering into philosophy.

The chief issue of the third group is raised by Prof. Arthur Bestor's contention that American schools are trying to do too much. If all three ends —skills, loyalties and intellectual discipline—are important, can a school sensibly try to achieve all of them? Perhaps a residential school can, since it has full control of the pupil's time. A day school cannot; if, with a few hours a day at its disposal, it tries to take over the functions once performed by home, church and work, as well as to discipline the young mind, it will do all of them badly. Prof. Bestor's proposal for American schools is clear-cut: let them realize that they are over-extending themselves and close their ranks, so that they can perform their own function well—that function being distinctively intellectual. To maintain their standards in the face of local pressures, they should set up State-wide systems of examination, which would free the able students from the educational 'lock step' and would test actual achievement rather than accumulations of credits and hours.

Prof. Bestor's views are challenged at several points by Prof. John L. Childs, who argues that the sensitivity of American schools to the social needs of their pupils is an advantage, not a weakness, and that the schools are not to be improved by a return to the narrow intellectualism of the French and other systems.

The issue raised by the two papers in the fourth group is: What can the schools do to prepare their

students for the sort of citizenship demanded by the United States' new place in the world? Dr. Reinhold Niebuhr tries to make this new place clear. It is one in which three new conditions prevail: America has become the acknowledged leader of the free world; is faced with a powerful and resolute rival; and must apparently live with a 'nuclear stalemate'. In this precarious position, technology is no doubt important; but Dr. Niebuhr thinks that an understanding of Russian imperialism and the curious American 'anti-imperialist imperialism' is equally so. It is necessary to have representatives abroad who speak the languages and understand the cultures of those among whom they are living; and though there is no study that will enable one to forecast the future, the study that does most to equip one for understanding the larger problems of the day is history.

Hans Morgenthau puts his emphasis in a different place. Agreeing that history is important, he thinks it could best be taught and understood not as a string of events but as the exemplification of principle, and above all, the principle that nations seek power. A thorough grasp of this principle, as exemplified historically, would throw more light on the present scene than any study of the events or personalities of the day.

The fifth group of articles examines the social matrix within which education in the United States operates. Prof. Margaret Mead looks into the reasons for the separation between the world of the scientist and that of his intended audience, the general population. In this way she points to the mechanism that may be responsible for the widening of the gap between the intellectual and society at large. Prof. David Riesman directs attention to the medium in which the academic himself grows and moves—lest educators seek the sources of their difficulties only outside the sphere of their own organizational setting.

Most of the papers have been edited by Prof. Brand Blanshard, who has also contributed an introduction to them. For background, this special issue of *Daedalus* also contains a reprint of A. N. Whitehead's famous essay on "The Aims of Education", which was first written some forty years ago. For those concerned with education, this essay may serve the purpose of periodic re-dedication as well as any other source.

PUBLIC LIBRARIES IN ENGLAND AND WALES

THE report of the Committee appointed by the Minister of Education in September 1957 to consider the structure of the public library service in England and Wales* reviews briefly the development of the public library service and the changing views of its functions, particularly the changes in formal structure since the Kenyon Committee reported in 1927. Turning then to the public library service to-day, it suggests that three main questions arise: how a really adequate service throughout the country can best be provided; the standards for judging the efficiency of a library service; and the requisite conditions which will enable a local authority to provide an efficient service. Chapters on the future structure of public library administration, library co-operation, staff and premises, the position in Wales, library charges and library legislation follow.

The principal recommendations are that the Minister of Education, assisted by two advisory bodies for England and Wales, respectively, should exercise a general responsibility for the public library service, with powers to enforce the discharge of its statutory duty by any library authority he considers to be in default. Every public library authority should have a statutory duty to provide an efficient library service, and the minimum annual expenditure on the purchase of books expected from a non-county borough or urban district council as an independent library authority should be £5,000 or 2*s*. per head of the population. All library authorities should be given powers to appoint library committees directly responsible to the councils.

The existing regional committees should be given statutory recognition and required, under schemes approved by the Minister, to provide a satisfactory system of library co-operation within their regions and to work in conjunction with the National Central Library. The local authorities should contribute substantially to the cost of the National Central Library, with greater representation on its governing body and in its administration. An adequate series of regional catalogues and the catalogue at the National Central Library should be completed as a matter of urgency and with a non-recurrent Treasury grant. Extra-district charges should be abolished for readers using libraries outside their own areas. Salary scales for the staff of public libraries should be commensurate with their qualifications and responsibilities and so applied as to make career prospects attractive. To meet the need for considerable improvement in public library premises and for many new library buildings a higher priority should be given to capital expenditure for this purpose. No charge should be made to borrowers of books or other material except for notification that the book or material is available or for retention beyond a prescribed period, and legislative provision should be made authorizing the levying of these charges by library authorities.

A new Public Libraries Act should be passed to give effect, so far as is necessary, to these recommendations and giving all library authorities specific powers to combine or co-operate with each other, and with education and other authorities, to provide lectures and cultural activities, to provide and lend such material as gramophone records and films and to acquire land compulsorily for library purposes under the Act. It is also recommended that the position of local authorities responsible for museum and art galleries should be considered, since it may be necessary to legislate separately for libraries, museums and art galleries in future. A minority report by Mr. J. Bulman and Mr. S. J. Skillen enters reservations as to the type of local authority which should become or remain library authorities and dissents from the suggestion as to a minimum expenditure on books.

* Ministry of Education. The Structure of the Public Library Service in England and Wales: Report of the Committee appointed by the Ministry of Education in September 1957. Pp. iv+57. (Cmnd. 660.) (London: H.M. Stationery Office, 1959.) 3*s*. 6*d*. net.

THE NATURE OF COAL

A SYMPOSIUM on the nature of coal was held at the Central Fuel Research Institute, Bihar, during February 7–9. The delegates were welcomed by Dr. A. Lahiri, director of the Institute, and by Prof. M. S. Thacker, director-general of scientific and industrial research in India, who referred to the valuable work already done by the Institute and to the need for symposia to review progress and exchange ideas. Dr. B. C. Guha of University College, Calcutta, gave the inaugural address. Coal, he said, was the parent of the industrial revolution and our principal source of heat and power. With the advent of atomic energy, some people feel that the use of coal as a fuel will decrease. Even if this were true, however, coal will still remain the source of numerous organic compounds—dyes, drugs, plastics and explosives. After less than a century of research on coal there remains much to be learnt and, no doubt, many new materials and processes to be developed.

The main subjects of the symposium were the origin and formation of coal; petrographic studies; X-ray structure; the constitution of coal as revealed by physical methods or by chemical methods; and the physico-chemical properties of coal. Contributions were received from a number of foreign countries, including Great Britain, the U.S.S.R., Japan, the United States, Canada, Australia, New Zealand, Czechoslovakia, Belgium, and West Germany.

In the session dealing with the origin and systematics of coal, the opening paper, by Dr. A. Lahiri, was on the metamorphism of coal, that is to say, on the transformation of vegetable matter and peat to lignite, bituminous coal and anthracite. This is referred to again later. In addition, there were interesting contributions from certain Russian investigators, namely, W. Rakovsky on the processes of formation of fuels and ways of solving the general problem of genesis, N. M. Karavayev on the early stages of formation of fossil fuel, A. Z. Yurovsky on the theory of the origin of sulphur in coals, V. S. Veselovsky on the chemical nature of caustobioliths from the point of view of genesis, and V. I. Kassatochkin on the physico-chemical aspects of the metamorphism of coals. There was also a well-discussed paper by A. Gillet and P. Colson on petroleum and anthracite from cellulose. Prof. Gillet, in presenting this paper, first dealt with the earlier work on the subject and later expounded his theories of the development of the main liquid and solid fuels from cellulose, employing numerous diagrams for illustration.

In the following session, papers were presented on petrographic studies on Indian coals and their relation to certain chemical properties, the significance of X-ray studies in the elucidation of problems of coal formation, deductions from recent X-ray data on coals, X-ray studies on oxidized coals and humic acids. On the ultra-fine structure of coal, the contributions were made on the sorption properties and structure, on the relation between internal surface and functional group measurements, on the adsorption of krypton by coal, and on the porosity of coal and of carbonized products as revealed by densities using helium and mercury as the displaced liquid. Considerable interest was shown in 'heat of wetting' and in the significance of the results obtained by different methods of determination.

In the papers and discussions on physical methods of studying the constitution of coals, the nine steps of Seyler did not receive support from T. N. Basu and D. Bagchi in their work on reflectance. Definite changes of reflectance, however, were confirmed at 85 per cent carbon and at 90–91 per cent carbon. Indeed, the changes when the carbon of coal exceeds 85 per cent were noted by many contributors from different sources. These changes are reflected in natural moisture, density, calorific value, oxygen, and coking properties, and seem to be related broadly to the elimination of hydroxyl groups in a drastic and abrupt manner involving re-orientation of molecular structure, probably with formation of quinone linkages. In India the mine dusts from such coals (85 per cent carbon) seem particularly dangerous in respect of mine explosions.

J. D. Brooks (Australia) presented a useful review of the principal chemical methods of investigating the constitution of coals, covering the results obtained by the various methods of determining functional groups, oxidation reactions, hydrogenation, hydrogenolysis, sulphonation, hydrolysis and pyrolysis of coals. J. B. Stott (New Zealand) dealt with the effects of evaporation on the early stage of spontaneous combustion of coal. Problems of coal heating have often presented baffling inconsistencies in the past: many of these could probably be explained by the influence of evaporation of water.

L. D. Ahuja and others, in discussing the conversion of non-coking coal to coking coal by partial hydrogenation, considered that the improvement in coking property is largely bound up with the removal of the more stable oxygen linkages. Drs. P. N. Mukherjee and A. Lahiri, dealing with the constitution of humic acids, pointed out that, on the basis of X-ray and infra-red studies, there is a close similarity between the structural units of coal and humic acids. From studies of various properties, including ultimate composition, molecular weight, equivalent weight and the distribution of functional groups, a tentative structural model was suggested for humic acids.

M. S. Iyengar and others claimed that with high-sulphur coals (up to 6 per cent organic sulphur) there are three different types of sulphur, namely: (1) sulphur eliminated during the initial stages of oxidation; (2) sulphur oxidized to sulphonic acids; and (3) sulphur resistant to oxidation. The first type has not been identified, the second has been identified as thiophenol, while the third is tentatively assumed to be thiophene.

A striking paper was given by B. K. Mazumdar and his colleagues on dehydrogenation studies carried out on several coals of different rank, on vitrains, on oxidized and weathered coals, and on coal extracts and pitch. They had used Vesterberg's technique. The optimum temperature for dehydrogenation with sulphur was found to be 200°–360° C. for bituminous coals and lignites. (Anthracite reacts with sulphur only above 480° C.) The hydrogen sulphide evolved accounts for more than 92 per cent of the products of reaction, but small amounts of methane are also detected. Dehydrogenation under these

conditions was ascribed primarily to the presence of alicyclic (hydro aromatic) structures, and the amount present was measured by the hydrogen sulphide formed. This was confirmed by a hydrogen balance of the coal and its dehydrogenated products. It was shown that 'alicyclicity' progressively diminished with rank, from 25 per cent in lignite to almost nil in anthracite. Similarly, alicyclicity with respect to hydrogen (Hal/H) varied from 60 per cent in lignite to nil in anthracite.

Further dehydrogenation of coal resulted in complete destruction of coking properties, inhibition of the formation of tar, and higher yields of char—apparently at the expense of the tar bodies. The yield of low-temperature tar was found to be proportional to (and also nearly equal to) the alicyclic carbon in coal.

Prior dehydrogenation led to the retention of 90 per cent of the original carbon of coal in low-temperature carbonization char, whether employing lignites or bituminous coals, whereas in normal (high-temperature) carbonization only 70–80 per cent of the carbon of the same coals was retained. The 'extra' carbon retained as a result of dehydrogenation and subsequent pyrolysis was considered to be a measure of the alicyclic carbon and hence of alicyclic hydrogen. A simple test of volatile matter in the presence of sulphur or selenium would afford a direct estimate of the alicyclic carbon retained in the char.

B. K. Mazumdar and his colleagues also explained the influence of the alicyclic component on the behaviour of coal in coking and tar formation, spontaneous combustion and weathering. Knowledge of the alicyclicity of coal enabled one to compute the aliphaticity, which was practically constant for all coals at 9–10 per cent of the carbon.

In a paper on aliphatic hydrogen in coal, J. N. Bhowmick and his collaborators described experiments employing chlorination to determine the aliphatic groups. Exhaustive chlorination of the coal at 200° C. was followed by hydrolysis in potassium hydroxide solution, and on the basis of the estimation of the functional groups formed in the hydrolysed products, as well as the loss of carbon sustained during chlorination, the distribution of aliphatic groups in coals of different ranks was worked out.

F. Kaiser, H. S. Rao and A. Lahiri, reporting on the disposition of methyl groups, described work in which they first formed an estimate of the side-chains in coal by the application of Kuhn–Roth's procedure for the determination of C–CH_3 groups. (The method depends on the fact that every side-chain contains one, and only one, methyl group, and that every methyl group can exist only as the terminal carbon of a side-chain.) The variation of C–CH_3 content with increase in rank was studied, and a free radical mechanism postulated for the coalification process. The mechanism attempts to explain two important facts associated with the metamorphism of coal, namely, the increase in aromaticity and the evolution of methane during the coalification process.

Dealing with pyrolysis and its relation to the structural parameters of coal, B. K. Mazumdar, S. K. Chakrabartty and A. Lahiri showed how, on a basis of recent knowledge obtained from X-ray and infra-red studies on carbonized coal (and in the light of oxidation and dehydrogenation studies on coal and its carbonized products), a mechanism could be worked out for the pyrolysis of coal up to 600° C. Low-temperature (600° C.) coke or char, they claimed, is entirely aromatic in character and no aromatic carbon of coal is lost by carbonization at 600° C. The primary bodies of tar come mainly from the alicyclic component, whereas the hydrocarbon gases come from the aliphatic structures. Correlation was shown to exist between the yield of gas and aliphaticity.

N. Berkowitz, in dealing with the decomposition and diffusion processes in coal pyrolysis, claimed that the classical interpretation attached to the weight-loss – temperature curve (namely, that it indicates a slow and extended chemical decomposition) is at variance with experimental facts. Such curves, if interpreted in the light of a diffusion process, offer material from which several important conclusions about the nature and kinetics of the pyrolytic processes can be drawn.

The broad picture that emerged from the papers and discussions was that coal can be regarded as essentially a binary mixture of aromatics dispersed in non-aromatics via carboxyl groups. Fusain (long attributed to forest fires) was probably one of the primary stages in coal formation.

Summarizing and following Lahiri on the metamorphism of coal, the general trend in the changes from peat to lignite, and bituminous coals, is primarily one of dehydration. At certain stages this dehydration seems to occur in discrete steps, notably at about 70, 85 and 91 per cent carbon. With increasing enrichment in carbon there is a diminution in the water-holding capacity of the material, representing a change from 'sol' to 'gel'. At the lignite stage there occurs an increase in hydroxyl groups, both alcoholic and phenolic, with some carboxyl groups, but quinone and carbonyl groups are relatively scarce. Some of the coal carbon—about 20 per cent—is present as alicyclic compounds, but about 60 per cent is in the form of aromatic compounds. This complex colloidal gel in lignite is still dispersed in water. The dispersing phase (water) is not merely absorbed physically in pores or micropores, but is largely held to the organic mass by chemisorption or hydrogen bonding and polar absorption. When the moisture falls to 15 per cent or so, the sub-bituminous or ligno-bituminous stage is reached, but the alicyclicity and aromaticity are largely unaffected. The oxygen and moisture in the dispersed phase are reduced, largely through dehydroxylation and decarboxylation, but dehydroxylation still continues up to 84–85 per cent carbon. Here, however, there is a marked change in capacity moisture and coking properties, reflected in changes of hardness, elasticity, calorific value and resistance to weathering. The reflectance and refractive index are also increased: alicyclic carbon increases, and also the yield of tar. Thus, the sol-to-gel transformation is complete and the stage of true bituminous coking coals reached. Under X-ray diffraction the structure of the coal showed an apparent emergence of order from previous disorder, but there is no definite evidence of any appreciable increase in the number of rings (averaging 4 or 5): the aromatic nucleus does not grow, although the coal becomes predominantly aromatic. Alicyclic carbon falls to 10 per cent of the coal, but is of great value in providing the 'coking constituents' of Bone and Fischer. Coking coals consist of aromatic ring structures dispersed in alicyclic carbon structures. In Nature such changes are apparently brought about by high tectonic pressures with considerable evolution of methane, especially when the carbon rises from 85 to 87 per cent.

Finally, in anthracites, the 'ordered' carbon is involved: there is an abrupt rise in reflectance and in aromaticity, with changes in general appearance

including the elimination of banding and petrographic heterogeneity. Coking properties are destroyed, and there is a small but definite rise in moisture content. The hydrogen content falls by 25–50 per cent of its earlier value. Apparently the organic gel has been transformed into an organic crystalloid. The process is thus primarily dehydration followed by decarboxylation, dehydroxylation and demethylation. The original aromatic carbon groups are not much affected by geochemical and geological changes until the 91 per cent carbon rank is reached. It is the variations in the alicyclic and aliphatic bodies which are largely responsible for the different types of coal, especially of bituminous coal.

The proceedings of the symposium are to be published in book-form. J. W. Whitaker

ROLE OF POLY-β-HYDROXYBUTYRIC ACID IN THE ASSIMILATION OF ORGANIC CARBON BY BACTERIA

By Prof. M. DOUDOROFF and Prof. R. Y. STANIER
Department of Bacteriology and Miller Institute, University of California, Berkeley

In 1936, Barker[1] observed that resting cells of a colourless alga, *Prototheca zopfii*, assimilate a large fraction of the carbon of the organic substrates which they oxidize. The same phenomenon was soon shown to occur in bacteria[2,3], and for some years the study of 'oxidative assimilation' had a considerable vogue among bacterial physiologists. The relevant literature has been reviewed by Clifton[4]. The discovery of the nature of bacterial photosynthesis by van Niel[5] in 1931 was followed by studies on the photosynthetic metabolism of organic substrates by the purple bacteria, which showed that under anaerobic conditions in the light these organisms can perform a very effective assimilation of organic carbon[6–8].

The work which has been done on the oxidative and photosynthetic assimilation of organic compounds by bacteria has thrown little light on the mechanism of the process. A dependence on the simultaneous formation of adenosine triphosphate is indicated by the observation that uncouplers of oxidative phosphorylation such as 2,4-dinitrophenol may prevent oxidative assimilation at levels which do not diminish the rate of respiration. From a study of the oxidative metabolism of specifically labelled radioactive substrates by *Pseudomonas saccharophila*, Wiame and Doudoroff[9] concluded that acetyl fragments are the intracellular metabolites that undergo assimilation.

It is furthermore evident that assimilation is not necessarily coupled with general cellular synthesis and growth, since bacteria can assimilate organic carbon with unimpaired efficiency in the absence of a nitrogen source. Under these conditions, the flow of carbon into the nitrogenous constituents of the cell must be negligible, and it could therefore be expected that the assimilated carbon accumulates in a limited group of cell materials. The evidence on this question is scanty and not clear. Gaffron, who first studied the photosynthetic assimilation of organic compounds by resting cells of purple bacteria[6,7], postulated the existence of a primary assimilatory product with the empirical formula $(C_4H_6O_2)_n$, and succeeded in isolating small quantities of a polymeric substance with this elementary composition from cultures grown photosynthetically on butyrate. Further pursuit of this lead seems to have been discouraged by van Niel's report[10] that the gross composition of the cells of purple bacteria corresponds closely to the composition of Gaffron's postulated assimilatory product, and the problem has remained where Gaffron left it in 1935. Inconclusive results have been obtained in the search for products of oxidative assimilation by resting bacteria. Wiame and Doudoroff[9] were unable to identify the radioactive cell-constituents formed by *Pseudomonas saccharophila* at the expense of substrates labelled with carbon-14, and suggested tentatively that the assimilated carbon flowed into many different cell materials, including proteins. Recently a similar conclusion was reached by Morita[11], who studied the dark oxidative assimilation of acetate, labelled with carbon-14, by *Rhodopseudomonas palustris*.

In 1927, Lemoigne[12] discovered that a polyester of β-hydroxybutyric acid is a major component of the cells of *Bacillus megaterium*, and the same substance was later found in other *Bacillus* species[13]. There is a considerable literature on the properties and metabolic behaviour of poly-β-hydroxybutyric acid in the genus *Bacillus*[13–20], and the accumulated evidence suggests that it functions as an intracellular reserve of carbon and energy for these bacteria. The recent observations of Macrae and Wilkinson[20] provide particularly convincing support for this hypothesis. The possible relevance of the investigations on poly-β-hydroxybutyric acid to the more general problem of bacterial oxidative and photosynthetic assimilation has been overlooked, no doubt because this polymer appeared until recently to be confined to a few species of bacteria. It was found in *Azotobacter* many years ago[21]; but its widespread occurrence in Gram-negative bacteria was first demonstrated in 1958[22]. It has not been detected in any other group of microorganisms, or in higher plants and animals.

The observation by our colleague Dr. Germaine Cohen-Bazire that purple bacteria accumulate massive quantities of poly-β-hydroxybutyric acid during photosynthetic growth on certain organic substrates prompted us to investigate its role in bacterial assimilation. We have examined the products of oxidative assimilation from glucose, acetate and butyrate in *Pseudomonas saccharophila*, and of photosynthetic assimilation from acetate and butyrate in *Rhodospirillum rubrum*. In all cases, a major fraction of the assimilated carbon (60–90 per cent) initially accumulates within the cell as poly-β-hydroxybutyric acid; when the external substrate is removed, there is a fairly rapid intracellular breakdown of the stored polymer. These observations suggest that poly-β-hydroxybutyric acid may have a general physiological function in bacteria as a primary product of carbon assimilation, its role in bacterial metabolism being analogous to that of

Table 1. PHOTO-ASSIMILATION OF LABELLED ACETATE AND BUTYRATE BY *Rhodospirillum rubrum*

	Substrate			
	0·01 *M* acetate		0·002 *M* butyrate	
	Cells	Polymer fraction	Cells	Polymer fraction
Initial dry weight (mgm.)	1,000	16	1,000	5
Increase in dry weight (mgm.)	585	329	260	130
Increase in polymer carbon* (mgm. C)		184		73
^{14}C incorporated (mgm. C)	248	173	100	58

* Calculated for $(C_4H_6O_2)_n$ from the increase in dry weight.

starch and glycogen in the metabolism of other organisms. Past failures to discover the polymer in bacteria (such as *P. saccharophila*) where it is a major component of the cell are readily understandable in view of its chemical properties. It represents a type of cellular constituent which is not detectable by the standard analytical procedures used for the fractionation of cell materials. Applied to cells which contain the polymer, these standard procedures result in the inclusion of the polymer with the protein fraction.

Isolation and Estimation of Poly-β-hydroxybutyric Acid

The poly-β-hydroxybutyric acid synthesized by *P. saccharophila* and *R. rubrum* is contained in sudanophilic cytoplasmic granules, similar to those of *Bacillus megaterium*[19]. These granules were isolated by digestion of the cells with alkaline hypochlorite[19], sedimented by centrifugation and washed with water. They were then treated with acetone and ether in order to remove lipids, after which the poly-β-hydroxybutyric acid was extracted with chloroform, dried and weighed. Such material is designated as 'polymer fraction' in the experiments described below. The poly-β-hydroxybutyric acid from each species was further purified by fractional precipitation from chloroform extracts with acetone/ether (2 : 1, v/v). The solubility properties, melting point and infra-red spectrum of the polymer from *P. saccharophila* and *R. rubrum* agree with those of the polymer from *Bacillus* spp.

Metabolic Role of Poly-β-hydroxybutyric Acid in *R. rubrum*

Polymer formation by *R. rubrum* at the expense of uniformly labelled acetate and butyrate was investigated. Cultures were grown at 30° C. in the light in Hutner basal medium[22] supplemented with either 0·01 *M* acetate or 0·005 *M* butyrate, and continuously aerated with 95 per cent nitrogen–5 per cent carbon dioxide. The cells were harvested by centrifugation, resuspended in Hutner phosphate buffer[24], and maintained in the light at 30° C. in a continuous stream of 95 per cent nitrogen to 5 per cent carbon dioxide for 12–16 hr., in order to deplete the internal polymer store. An initial sample was then taken, radioactive substrate was introduced, and the aeration mixture was changed to 95 per cent helium–5 per cent carbon dioxide in order to prevent nitrogen fixation. When most of the radioactive substrate had disappeared from the suspending medium, the cells were harvested by centrifugation, washed and resuspended in a small volume of distilled water. Dry weights of cells and polymer were measured on initial and final samples, and radioactivities on the final sample. The results are shown in Table 1. For ease of comparison, the actual data have been recalculated on the basis of an initial dry weight of cells of 1,000 mgm. In the experiment on acetate assimilation, the cellular dry weight increased by 594 mgm., and the polymer fraction accounted for 329 mgm. of this increase. The quantity of polymer carbon in the cells increased by 184 mgm., and the weight of carbon-14 incorporated from acetate in the polymer fraction was 174 mgm. From this it follows that acetate carbon flows into polymer without appreciable dilution, either by pre-existing cellular carbon, or by fixation of carbon dioxide. The experiment on assimilation of butyrate gave similar results; but somewhat less of the assimilated butyrate carbon was incorporated in polymer (58 per cent of the total, in contrast to nearly 70 per cent of the total in the case of acetate), and the dilution of radioactivity was appreciably greater.

The cells which had become radioactive as a result of assimilating labelled acetate or labelled butyrate were used for experiments on the subsequent fate of the polymer. The washed cells were resuspended in Hutner phosphate buffer supplemented with 0·1 per cent ammonium chloride, and illuminated for 12 hr. without an exogenous organic carbon source in a constant stream of 95 per cent nitrogen and 5 per cent carbon dioxide. Additional aliquots of the cells labelled from butyrate-^{14}C were similarly treated, but were furnished with an exogenous organic carbon source (either butyrate or succinate). At the end of the period of illumination, the cells were harvested, and radioactivities and dry weights of the cells and the polymer fraction were again determined. The results are shown in Table 2. In the absence of an exogenous organic carbon source, more than 90 per cent of the stored polymer disappeared, but a substantial part of the carbon-14 initially present in the polymer fraction remained in the cells. This fact shows that the polymer can serve as an endogenous carbon source for the synthesis of other cell-constituents. The cells provided with an exogenous

Table 2. FATE OF POLY-β-HYDROXYBUTYRIC ACID IN *Rhodospirillum rubrum* INCUBATED ANAEROBICALLY IN THE LIGHT WITH A NITROGEN SOURCE, IN THE PRESENCE AND ABSENCE OF EXOGENOUS SUBSTRATES

Experiment No.	1		2					
Exogenous carbon source	None		None		Butyrate		Succinate	
	Cells	Polymer	Cells	Polymer	Cells	Polymer	Cells	Polymer
Initial dry wt. (mgm.)	1,000	213	1,000	108	1,000	108	1,000	108
Change in dry wt. (mgm.)	−50	−201	−91	−100	+322	+13	+377	−96
Initial ^{14}C (mgm. C)	156	110	78	46	78	46	78	46
Change in ^{14}C (mgm. C)	−48	−106	−31	−43	−17	−14	−29	−44
Net ^{14}C transfer from polymer to other cell constituents (mgm. C)	58		12		nil		15	

Exp. 1 performed with cells which had assimilated labelled acetate. Exp. 2, with cells which had assimilated labelled butyrate.

supply of butyrate increased in dry weight by about 30 per cent, and in polymer content by about 10 per cent. In this case, the loss of carbon-14 from the polymer fraction was relatively small, and almost identical with the total loss of carbon-14 from the cells. It is thus evident that the continued presence of exogenous butyrate greatly reduces the internal breakdown of polymer, and impedes its transfer to other cell-constituents. The effect of an exogenous supply of succinate is strikingly different. Under anaerobic conditions in the light, succinate is not used by *R. rubrum* for polymer synthesis, although it is an excellent substrate for growth. The cells furnished with succinate increased their dry weight by almost 40 per cent; but the succinate conferred no protection on the polymer pool, which disappeared to the same extent as in cells not furnished with an exogenous organic carbon source, and with a similar amount of transfer to other cell-constituents.

Metabolic Role of Poly-β-hydroxybutyric Acid in *P. saccharophila*

Washed cells of *P. saccharophila* strain *G* grown on mineral-glucose medium[25] were resuspended in 0·03 *M* phosphate buffer (*p*H 6·8). An initial sample was taken, uniformly labelled glucose-^{14}C at a final concentration of 0·4 per cent was added, and the suspension was shaken in air at 30° C. for 100 min. During this time, almost half the glucose was metabolized, and about 21 per cent of the glucose carbon which disappeared from the medium was incorporated in the cells. The cells were harvested and analysed as already described for *R. rubrum*. The results (Table 3) show that 66 per cent of the glucose carbon assimilated was incorporated in the polymer fraction, and that glucose flowed into polymer with very little dilution of specific activity.

Table 3. Oxidative Assimilation of Labelled Glucose by *Pseudomonas saccharophila*

	Cells	Polymer fraction
Initial dry weight (mgm.)	1,000	144
Increase in dry weight (mgm.)	193	144
Increase in polymer carbon* (mgm. C)		81
^{14}C incorporated (mgm. C)	112	74

* Calculated for $(C_6H_8O_2)_n$ from the increase in dry weight.

The assimilation of glucose observed in this experiment was much less than that customarily observed in manometric experiments, conducted with starved cells which are allowed to metabolize very small quantities of substrate[26,27]. Accordingly, a series of experiments on polymer formation under conditions comparable to those employed in the earlier manometric studies on assimilation by *P. saccharophila* were undertaken. Cells were grown on media with a mineral base, supplemented with the carbon source to be used in the subsequent experiment. They were harvested, resuspended in phosphate buffer, and shaken in air for 16 hr. at 30° C. in order to deplete their endogenous reserves. These starved cells were then permitted to oxidize a small quantity of substrate uniformly labelled with carbon-14, the course of the oxidation being followed manometrically. As soon as the uptake of oxygen indicated that substrate utilization was approaching completion, the cells were chilled to 0° C., centrifuged, washed in the cold, and resuspended in distilled water. An aliquot was used for the determination of the total radioactivity of the cells, and the remainder was digested with alkaline hypochlorite. The polymer was extracted from this digest by shaking twice with chloroform, and 10 mgm. of purified polymer was added to the chloroform extract to serve as carrier. The extract was then washed with water and evaporated to dryness. The residue was redissolved in hot chloroform, and the polymer was precipitated from this solution by the addition of an acetone–ether mixture. The amount of polymer synthesized was calculated from the radioactivity of the polymer so recovered. The results (Table 4) show that a large part of the carbon assimilated from glucose, acetate and butyrate under conditions where the efficiency of assimilation is very high is incorporated in the polymer fraction.

Table 4. Oxidative Assimilation of Small Quantities of Glucose, Acetate and Butyrate, Uniformly Labelled with Carbon-14, by Starved Cells of *Pseudomonas saccharophila*

	Substrate furnished		
	Glucose-^{14}C 5 μmoles	Acetate-^{14}C 20 μmoles	Butyrate-^{14}C 10 μmoles
Substrate metabolized, μatoms of C	24·2	38·4	33·4
Substrate assimilated by cells, μatoms of C	14·4	17·5	20·8
Substrate incorporated in polymer fraction, μatoms of C	9·2	14·4	16·7

Other tracer experiments have shown that the polymer can serve as a substrate for endogenous metabolism in *Pseudomonas saccharophila* in the absence of an exogenous carbon source. It is, however, much more slowly metabolized than in *R. rubrum* and a net transfer of polymer carbon to other cell constituents has not yet been demonstrated.

We wish to acknowledge the valuable assistance of Miss Riyo Kunisawa and Mrs. Rebecca Contopoulou in the performance of the experiments. Radioactive substrates were kindly furnished by Prof. H. A. Barker. The work forms part of a programme supported by a grant from the National Institutes of Health.

[1] Barker, H. A., *J. Cell. Comp. Physiol.*, **8**, 231 (1936).
[2] Giesberger, G., "Beiträge zur Kenntnis der Gattung Spirillum", Dissertation (Utrecht, 1936).
[3] Clifton, C. E., *Enzymolog.*, **4**, 246 (1937).
[4] Clifton, C. E., "Adv. in Enzymol.", **6**, 269 (1946).
[5] van Niel, C. B., *Arch. Mikrobiol.*, **3**, 1 (1931).
[6] Gaffron, H., *Biochem. Z.*, **260**, 1 (1933).
[7] Gaffron, H., *Biochem. Z.*, **275**, 301 (1935).
[8] Muller, F. M., *Arch. Mikrobiol.*, **4**, 131 (1933).
[9] Wiame, J. M., and Doudoroff, M., *J. Bact.*, **62**, 187 (1951).
[10] van Niel, C. B., *Arch. Mikrobiol.*, **7**, 323 (1936).
[11] Morita, S., *J. Biochem.* (Japan), **45**, 651 (1958).
[12] Lemoigne, M., *Ann. Inst. Pasteur*, **41**, 148 (1927).
[13] Lemoigne, M., *Helv. Chim. Acta*, **29**, 1303 (1946).
[14] Lemoigne, M., and Roukhelman, N., *Ann. Ferment.*, **5**, 527 (1940).
[15] Lemoigne, M., Delaporte, B., and Croson, M., *Ann. Inst. Pasteur*, **70**, 244 (1944).
[16] Lemoigne, M., Grelet, N., and Croson, M., *Bull. Soc. Chim. Biol., Paris*, **32**, 719 (1950).
[17] Lemoigne, M., Grelet, N., Croson, M., and Le Treis, M., *Bull. Soc. Chim. Biol., Paris*, **27**, 90 (1945).
[18] Képès, A., and Péaud-Lenoël, C., *C.R. Acad. Sci., Paris*, **234**, 756 (1952).
[19] Williamson, D. H., and Wilkinson, J. F., *J. Gen. Microbiol.*, **19**, 198 (1958).
[20] Macrae, R. M., and Wilkinson, J. F., *J. Gen. Microbiol.*, **19**, 210 (1958).
[21] Lemoigne, M., and Girard, H., *C.R. Acad. Sci., Paris*, **217**, 557 (1943).
[22] Forsyth, W. G. C., Hayward, A. C., and Roberts, J. B., *Nature*, **182**, 800 (1958).
[23] Cohen-Bazire, G., Sistrom, W. R., and Stanier, R. Y., *J. Cell. Comp. Physiol.*, **49**, 25 (1957).
[24] Jensen, S. L., Cohen-Bazire, G., Nakayama, T. O. M., and Stanier, R. Y., *Biochim. Biophys. Acta*, **29**, 477 (1958).
[25] Doudoroff, M., Palleroni, N. J., MacGee, J., and Ohara, M., *J. Bact.*, **71**, 196 (1956).
[26] Doudoroff, M., *Enzymology*, **9**, 59 (1940).
[27] Whelton, R., and Doudoroff, M., *J. Bact.*, **49**, 177 (1945).

A NEW SHORT-ACTING THIOBARBITURATE

By Dr. M. E. PEEL, BARBARA MACAULEY*, Dr. H. O. J. COLLIER†, Dr. J. H. HUNT, Dr. F. A. ROBINSON and Dr. E. P. TAYLOR

Research Division, Allen and Hanburys, Limited, Ware, Herts

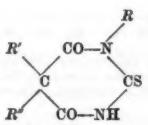
OF the thiobarbiturates used as intravenous anæsthetics, thiopentone, introduced in 1934, has been in recent years the most widely used. Figures for 1955 show that it was employed "in over 80 per cent of all anæsthetics in Great Britain"[1].

Unfortunately, while thiopentone produces anæsthesia of brief duration, considerable time may elapse following recovery of consciousness before the mental faculties of the patient return completely to normal, which is a disadvantage in some circumstances. Indeed, Dundee[2] states that "All out-patients who are to receive thiopentone must have the hazards explained to them beforehand and an escort for the journey home must be insisted upon. Unless arrangements are made for this escort or the patient is willing to spend the night in hospital, thiopentone should not be given".

acids were therefore prepared by conventional methods, for example, by the condensation of N-alkyl thioureas with either (*a*) the appropriate cyanoacetic ester (with subsequent hydrolysis) or (*b*) the appropriate malonic ester.

These compounds were examined pharmacologically by a step-wise procedure designed to screen for potency, safety and brevity. Each compound was administered intravenously to mice, and their responses were determined on the rotating drum[5]. The median effective dose (*ED*50) so found was then compared with the median lethal dose (*LD*50) to give an approximate therapeutic index. When this index permitted, the duration of action was measured at doses effective in all animals but lethal to few or none. Results of such experiments with some of these compounds are summarized in Table 1.

Table 1. Potency, Toxicity and Duration of some Thiobarbituric Acids in Mice
Values are expressed in mgm./kgm. acid with 0·95 fiducial limits



Compound	*R'*	*R''*	*R*	*ED*50 (fiducial limits)	*LD*50 (fiducial limits)	*LD*50/*ED*50	Approximate duration at 7 × *ED*50 (sec.)
Thiopentone	1. Me–Bu^n	Et	H	5·98 (4·44–6·95)	70·25 (61·76–87·10)	12·08	3,500
Buthalitone	Bu^i	Allyl	H	17·25 (15·30–18·80)	149·9 (106·9–158·2)	8·69	20,000
B.82	Bu^i	Allyl	Me	9·57 (9·05–10·12)	81·96 (76·54–87·76)	8·56	550
B.116	Bu^i	Allyl	Pr^n	24·11 (20·09–28·92)	103·50 (96·36–111·10)	4·29	—
B.117	1. Me–Bu^n	Allyl	Bu^n	20·40 (15·42–26·99)	76·43 (61·60–94·84)	3·75	—
B.133	Bu^i	Et	Me	7·33 (6·57–7·95)	76·17 (69·76–80·98)	10·39	600
B.86	Bu^i	Et	Et	13·21 (11·59–15·07)	93·17 (86·94–99·86)	7·05	—
B.137*	Bu^i	Et	Me	7·38 (6·31–8·22)	83·60 (80·65–87·06)	11·33	550
B.135	Bu^s	Allyl	Me	6·05 (5·25–6·72)	73·38 (70·55–76·17)	12·13	450
B.80	Bu^s	Allyl	Bu^n	14·14 (10·30–19·40)	91·33 (86·60–96·32)	6·46	—
B.139	Bu^i	Pr^n	Me	11·68 (10·22–13·32)	71·06 (64·34–78·50)	6·08	—
B.158*	Bu^s	Et	Me	6·79 (5·97–7·72)	73·86 (70·30–77·82)	10·88	1,000

* Racemic stereoisomers.

Following a suggestion made by Dr. J. G. Bourne, a search was initiated for an intravenous anæsthetic from which recovery would be more rapid than from thiopentone.

It is known[3,4] that N-alkylation of 5 : 5-disubstituted thiobarbituric acids tends to shorten the duration of action, and accordingly some 150 new N-alkyl thiobarbituric acids were synthesized.

The alkylation of 1 : 5-dialkyl thiobarbituric acids was first investigated, but this resulted in S-alkylation with the formation of the apparently unrecorded 2-alkylmercapto-1 : 5-dialkyltetrahydropyrimidine-4 : 6-diones. The required 1 : 5 : 5-trialkyl thiobarbituric

From Table 1 it will be seen that the ratio *LD*50/*ED*50 falls as the size of the alkyl group on the nitrogen atom increases (for example, buthalitone, B.82, B.116; B.133, B.86; B.135, B.80). The greater brevity of B.82 compared with buthalitone illustrates the rule that methylation of the nitrogen atom tends to shorten the duration of action.

Fig. 1 illustrates the relation of dose to duration of narcosis with those compounds in Table 1 in which the index *LD*50/*ED*50 is high. With B.82 the line relating duration of action to dose remained straight even when carried into the range where some doses were lethal. With buthalitone this line turned sharply upwards long before lethal doses were reached, and higher doses gave narcosis too prolonged to be accommodated on the scale of the graph (see Table 1). The

*Present address: Pharmacology Department, Albert Einstein College of Medicine, Yeshiva University, New York, U.S.A.
† Present address: Department of Pharmacological Research, Parke, Davis and Co., Ltd., Hounslow, Middlesex.

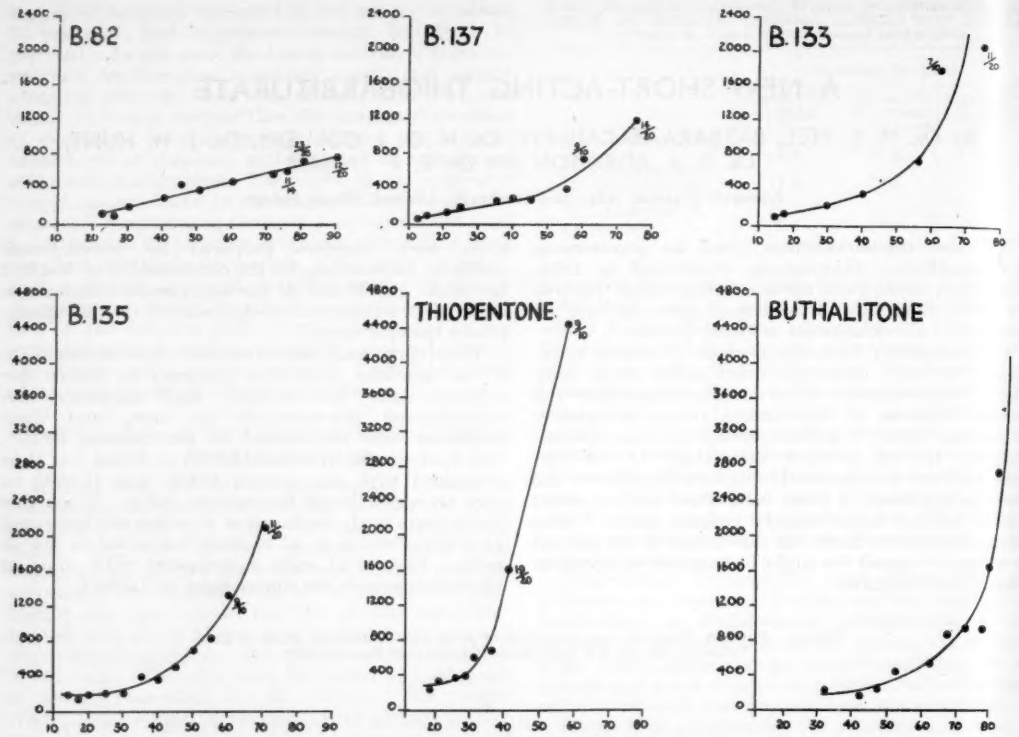


Fig. 1. Relation of dose to duration of thiobarbiturates administered intravenously to mice. All doses were effective in all mice. Where some mice died, the proportion of survivors is indicated. Ordinates, mean duration in sec.; abscissæ, intravenous dose in mgm./kgm.

curves for other compounds were intermediate in slope between those of B.82 and of buthalitone. B.158 is not included in Fig. 1, but it had a curve comparable to that of thiopentone. The flatness of the curve at high doses was taken as a favourable feature in selecting compounds for further study.

A similar procedure was adopted for selected compounds in rabbits except that loss of righting response was used instead of the rotating drum as a test of effectiveness. Some compounds were also administered intravenously to cats in order to observe the effect on blood pressure and cardiac rhythm. Compounds which possessed potency and safety comparable to thiopentone and which were considerably briefer in duration were examined by further tests, which included cumulative action and depression of respiration in rabbits, depression of ganglionic transmission in cats, local and sub-acute toxicities and duration of action after experimental liver or kidney damage in mice.

Members of the S-alkyl group had low therapeutic indices in mice and caused cardiac arrhythmias in cats. These compounds were therefore not pursued further.

The properties of three of the compounds in Table 1 (B.82, B.133 and B.137) appeared sufficiently promising in animals to warrant their preliminary investigation in man. B.82 and B.133 were soon discarded owing to the incidence of muscular tremor, sneezing or hiccough (Bourne, J. G., private communication). B.137, however, did not show these undesirable side-effects, and in a series of 71 cases receiving identical doses of either B.137 or thiopentone, no variation was observed in reactions or the time of the induction period; surgical anæsthesia was equally satisfactorily produced by either drug. Moreover, the time of recovery following administration of B.137 was approximately half that required for recovery from thiopentone (Day, D., private communication). Similar results were obtained in a smaller trial of 12 cases (Rees, L. T., private communication). Wider clinical investigations are now in progress.

The potency, toxicity and duration of B.137 in rabbits are compared with those of thiopentone in Table 2.

Table 2. Potency, Toxicity and Duration of B.137 and Thiopentone in Rabbits
Values are expressed in mgm./kgm. ± standard error

Compound	*ED*50	*LD*50	$\frac{LD50}{ED50}$	Approximate duration at 7 × *ED*50 (sec.)
Thiopentone	2·03 ± 0·26	26·51 ± 0·42	13·06	1,200
B.137	2·12 ± 0·24	29·73 ± 0·83	14·02	709

B.137, a white crystalline solid (m.p. 96–97° C.), is the higher melting of the two possible racemic forms of 1-methyl-5-*sec*butyl-5-ethyl-thiobarbituric acid: B.158 (m.p. 56–57° C.), the other form, has been separated and also independently synthesized.

Like thiopentone, B.137 is best administered as its sodium salt, and is presented as a sterile mixture of this salt with a suitable buffer. The chemistry and pharmacology will be described in further detail elsewhere.

We thank Dr. J. G. Bourne for his valuable advice, Mr. R. T. Brittain for some experimental contributions, Miss J. Heathcote for assistance in the pharmaceutical presentation, Mr. J. J. Grimshaw for statistical analysis, Mrs. E. Nield, Miss A. Stripling and Mr. S. Ward for technical assistance, and the Directors of Allen and Hanburys, Limited, for permission to publish this work.

[1] Dundee, J. W., "Thiopentone and other Thiobarbiturates", 10 (E. and S. Livingstone, Ltd., Edinburgh and London, 1956).
[2] Dundee, J. W., "Thiopentone and other Thiobarbiturates", 216 (E. and S. Livingstone, Ltd., Edinburgh and London, 1956).
[3] Swanson, E. E., and Chen, K. K., *Proc. Soc. Exp. Biol., N.Y.*, **82**, 212 (1953).
[4] Stoelting, V. K., and Graf, J. P., *Anesthesiology*, **15**, 61 (1954).
[5] Collier, H. O. J., Hall, R. A., and Fieller, E. C., *Analyst*, **74**, 592 (1949).

AMINO-ACID COMPOSITION OF THE PROTEINS OF BARLEY LEAVES IN RELATION TO THE MINERAL NUTRITION AND AGE OF PLANTS

By Dr. B. P. PLESHKOV* and Dr. L. FOWDEN†

It is well known that the total nitrogen content and the relative amounts of different nitrogenous fractions of plants vary with their age and conditions of mineral nutrition. Particular attention has been paid to the free amino-acid fraction; in general the content of free amino-acids in plants increased under conditions of potassium[1–3], sulphur[4,5], calcium, magnesium and boron[6], and zinc, copper, manganese and iron[7–9] deficiencies. A similar increase results when plants are supplied with abundant nitrogen[2,10] or chlorine[11]. Nitrogen and molybdenum deficiencies caused a decrease in the free amino-acid content[2,3,8,10]. The free amino-acid content of the vegetative organs generally decreases with increasing age of the plant[5,10].

The amino-acid composition of the proteins of the vegetative organs and the changes of composition caused by different environmental factors have been studied to a lesser extent. Recent results suggest that the amino-acid composition of the proteins of the active vegetative organs is approximately constant. The investigations of Fowden showed that a close similarity exists between the composition of proteins isolated from different algae[12] and that the composition of *Chlorella* proteins does not change significantly with age[13]. Yemm and Folkes[14] did not find any significant differences between the amino-acid composition of the cytoplasmic and chloroplastic proteins of barley leaves, while Bryant and Fowden[15] detected only small differences in the amino-acid composition of the proteins extracted from daffodil leaves of different ages. This constancy of composition is not absolute, and results have been obtained indicating that the contents of individual amino-acids in the proteins isolated from sugar beet of different ages vary considerably[16]. No observations appear to exist relating plant protein composition with the conditions of mineral nutrition under which the plant developed.

The aim of the present work was to study the content of the free amino-acids and the amino-acid composition of the proteins of barley leaves of different ages grown under different nutrient conditions in respect to nitrogen, phosphorus and potassium. The barley (variety *B*-2118) was grown in sand culture in Richards's nutrient solution[17] either complete or at one-tenth dilutions in respect of nitrogen, phosphorus and potassium separately. Samples were collected 24 and 48 days after germination of the plants; the whole of the aerial portion of the plants was taken for analysis. Leaf proteins were extracted and hydrolysed by the method described earlier[18]. Free amino-acids were extracted with 80 per cent ethanol from individual plant samples, and each acid was determined quantitatively by paper chromatography[15]. Tryptophan was determined by the method of Spies and Chambers[18] (procedure K) and proline by Chinard's method[19].

The total nitrogen, protein nitrogen and amino-acid concentrations of the plants are given in Table 1. Total and protein nitrogen decreased with increasing age of plant. Considerable differences were also detected between plants receiving different treatments. Nitrogen deficiency caused a striking decrease in total and protein nitrogen; in potassium-deficient plants the total nitrogen concentration was practically unchanged, but protein nitrogen decreased markedly, especially in plants 48 days old; phosphorus deficiency also reduced the total and protein nitrogen of the plants.

In plants grown under normal nutrient conditions the total free amino-acid concentration decreased noticeably with age. All amino-acid concentrations were reduced in approximately equal proportions except those of aspartic acid and alanine, which were decreased more markedly than the rest. A slight increase in the concentration of γ-amino-butyric acid was observed. After only 24 days, nitrogen deficiency had caused a large decrease, relative to the control plants, in the concentrations of all amino-acids except tyrosine and proline. The tyrosine-level remained approximately unchanged while proline was increased three-fold. In 48-day-old nitrogen-deficient plants, total free amino-acids decreased to a still lower level. The further reduction affected mainly aspartic and glutamic acids, serine, glutamine, asparagine and proline. Only the pipecolic acid concentration

Table 1. Nitrogen and Amino-acid Content in Plants (per cent of the dry matter)

Age of plants	Treatment	Total nitrogen	Protein nitrogen	Free amino-acids (total)
24 days	NPK	4·86	3·85	2·34
	N(1/10)PK	3·22	2·75	1·39
	P(1/10)NK	4·32	3·04	2·38
	K(1/10)NP	4·56	3·71	2·19
48 days	NPK	3·85	2·64	1·59
	N(1/10)PK	1·47	1·21	0·52
	P(1/10)NK	3·46	2·42	2·34
	K(1/10)NP	3·78	2·14	2·22

* Exchange fellow sponsored by the British Council. Permanent address: Department of Agricultural and Biological Chemistry, Timiryazev Agricultural Academy, Moscow, A8; now at Rothamsted Experimental Station, Harpenden, Herts.
† Department of Botany, University College, London, W.C.1.

Table 2. Amino-acid Content of the Protein Preparations
(Amino-acid nitrogen as percentage of protein nitrogen)

	24 days				48 days			
	NPK	N(1/10)PK	P(1/10)NK	K(1/10)NP	NPK	N(1/10)PK	P(1/10)NK	K(1/10)NP
Cysteine + cystine	trace*	trace*	trace*	trace*	trace*	trace*	trace*	trace*
Leucines	12·2	12·3	13·1	12·6	13·4	11·9	13·3	12·6
Arginine	13·0	13·5	13·5	13·4	11·9	11·9	9·9	10·6
Histidine	3·8	3·5	3·5	4·8	4·2	2·3	3·3	4·0
Lysine	5·3	5·6	6·0	5·3	7·6	7·1	6·0	6·3
Aspartic acid	7·1	6·2	6·9	7·1	7·2	6·9	6·1	7·6
Glutamic acid	7·5	7·6	7·5	6·3	7·0	6·7	6·0	7·0
Serine	2·9	2·7	2·9	2·5	2·4	2·9	2·1	2·5
Glycine	6·8	7·1	7·1	6·3	6·7	6·8	6·7	6·3
Threonine	3·6	3·8	3·8	3·4	3·5	3·7	2·6	2·9
Alanine	5·9	7·7	7·7	7·6	6·8	7·2	6·5	6·7
Valine	6·0	6·1	6·6	6·4	7·8	6·1	6·5	6·3
Tyrosine	2·1	2·6	3·1	2·8	3·2	3·5	2·4	3·0
Methionine	2·7	2·1	1·9	2·1	1·9	2·0	2·5	2·3
Proline	5·1	5·8	5·4	4·2	4·1	5·4	4·6	4·0
Phenylalanine	3·2	3·6	2·8	2·7	3·3	3·8	3·0	2·9
Tryptophan	1·9	1·3	1·3	1·2	1·4	1·0	1·4	1·2
Unknown amino-acid†	2	2	1	1	3	2·8	2·4	2
Amides	5·8	3·4	5·7	5·6	4·0	3·6	3·8	4·7
Total	96·9	96·9	99·8	95·3	99·4	95·6	89·1	92·9

* Less than 0·5 per cent.
† Approximate determinations.

remained unchanged when compared with control plants. Phosphorus- and potassium-deficient plants contained approximately the same total amounts of free amino-acids as control plants at the 24-day stage; at 48 days these totals remained unaltered for the deficient plants, although the level of free amino-acids in control plants had decreased. Variations in the amounts of certain individual amino-acids relative to the controls served to characterize the deficient plants. Phosphorus deficiency was associated with a decrease in the concentration of glutamic acid and a corresponding increase in glutamine, the concentration of which was five times that of normal plants at the 48-day stage. Unusually high pipecolic acid concentrations were also associated with phosphorus deficiency. Potassium deficiency was characterized mainly by a slight decrease of the concentrations of glutamic and aspartic acids relative to control plants and a marked increase in asparagine concentrations. At 48 days, one-third of the total nitrogen of the free amino-acids was in the form of this amide.

The amino-acid compositions of the protein preparations from the corresponding plants are given in Table 2. Although marked differences occurred in the total and protein nitrogen-levels of the plant tissues of different age and nutritional status, the amino-acid composition of their proteins showed distinct similarity; the values obtained for many amino-acids do not differ significantly for the various treatments if allowance is made for an experimental error of ± 5–7 per cent of the reported values. There is a trend in the proteins of older plants towards decreasing amide- and arginine-levels and slightly increased lysine contents. Nitrogen deficiency produced a definite decrease in the amide content at 24 days, and in histidine at 48 days. Smaller changes in the contents of threonine, alanine, methionine, arginine, proline and tryptophan also occurred as the nutrient conditions were varied.

Actively growing vegetative plant organs contain a large number of different enzymic proteins having different amino-acid compositions; at various stages of plant development, and under various environmental and nutritional conditions, the relative amounts of these proteins may vary widely. However, analyses of 'bulk' proteins show only their mean amino-acid composition, and real differences in concentrations of individual proteins can become masked when analyses are portrayed in terms of 'bulk' protein composition. The similarity of the amino-acid compositions determined for the proteins of plants grown under different nutrient regimes can then be still compatible with an altered balance in the complement of individual proteins.

Analysis of the barley proteins consistently revealed a new unknown amino-acid in addition to the amino-acids characteristic of proteins. The R_F of this amino-acid in butanol/acetic acid/water solvent[20] was approximately half that of cysteic acid, and so it was the slowest-moving component of the protein hydrolysate. It was only possible to detect this substance after chromatograms had been developed for at least 6 days. A few milligrams of partially purified material has been isolated, and by means of electrophoresis it has been shown to have a slightly acidic character. The values given in Table 2 for the content of this amino-acid in the protein preparations are calculated assuming a molecular weight of 75; that is, the same as glycine. The amounts of the amino-acid contained in the proteins definitely increased with the age of the plant. Isolation and identification of this amino-acid will be a problem for future investigation.

One of us (B. P.) wishes to thank Dr. G. W. Cooke for his advice and help during these investigations.

[1] Richards, F. J., and Berner, E., *Ann. Bot.*, N.S., **17**, 15 (1954).
[2] Dadykin, V. P., and Igumnova, G. S., *Fisiol. Rastenii*, **3**, 431 (1956).
[3] Pleshkov, B. P., Ivanko, Sh., and Antonova, G. V., *Doklady Akad. Nauk. S.S.S.R.*, **117**, 1070 (1957).
[4] Mertz, E. T., and Matsumoto, H., *Arch. Biochem. Biophys.*, **63**, 50 (1956).
[5] Coleman, R. G., *Austral. J. Biol. Sci.*, **10**, 50 (1957).
[6] Steinberg, R. A., Bowling, J. D., and McMurtry, J. E., *Plant Physiol.*, **25**, 279 (1950).
[7] Hewitt, E. J., Jones, E. F., and Williams, A. H., *Nature*, **163**, 681 (1949).
[8] Possingham, J. V., *Austral. J. Biol. Sci.*, **9**, 539 (1956); **10**, 40 (1957).
[9] DeKock, P. C., and Morrison, R. J., *Biochem. J.*, **70**, 266 (1958).
[10] Pleshkov, B. P., Shmyreva, T. V., and Ivanko, Sh., *Fisiol. Rastenii*, **6** (1959) (in the press).
[11] Noboru, S., and Akira, M., *J. Sci. Soil and Manure* (Japan), **27**, 215 (1956).
[12] Fowden, L., *Ann. Bot.*, N.S., **18**, 257 (1954).
[13] Fowden, L., *Biochem. J.*, **50**, 355 (1952).
[14] Yemm, E. W., and Folkes, B. F., *Biochem. J.*, **55**, 700 (1953).
[15] Bryant, M., and Fowden, L., *Ann. Bot.*, N.S., **23**, 65 (1959).
[16] Sissakyan, N. M., Bezinger, E. N., Gumilevskaya, N. A., and Lukyanova, F. J., *Biokhimiya*, **20**, 368 (1955).
[17] Richards, F. J., and Shin, Sheng-Han, *Ann. Bot.*, N.S., **4**, 165 (1940).
[18] Spies, J. R., and Chambers, D. C., *Anal. Chem.*, **21**, 1249 (1949).
[19] Chinard, F. P., *J. Biol. Chem.*, **199**, 91 (1952).
[20] Partridge, S. M., *Biochem. J.*, **42**, 238 (1949).

LETTERS TO THE EDITORS

RADIOBIOLOGY

Uptake of Strontium by Pasture Plants and its Possible Significance in Relation to the Fall-out of Strontium-90

THE recognition of the importance of the chain, plant–animal–man[1], in the uptake of strontium-90 by humans as a result of the fall-out of fission products made it desirable to see if species differences existed in forage plants, such that some might take up less strontium from the soil than others. This knowledge might then be used to reduce the radiostrontium content of milk, or to choose species for the reclaiming of contaminated land.

Sixteen species and strains of forage plants were grown in 1-gallon stone crocks on three different soils, similar in texture and *p*H but varying in strontium and calcium content (Table 1). Neither radioactive nor stable strontium was added to the soil, because of the difficulties of mixing and because the very small amounts of native strontium should give a better picture of the uptake of minute amounts of strontium by plants. Harvesting was carried out at eight weeks, when the plants reached the stage of incipient flowering or had reached maximum vegetative growth.

Table 1. STRONTIUM AND CALCIUM CONTENT AND *p*H OF THE SOILS USED IN THE EXPERIMENT

Soil	Calcium (μeq./100 gm.)	Strontium (μeq./100 gm.)	Calcium/strontium ratio	*p*H
Nord fine sandy loam	17,260	52·8	342	7·4
Yolo fine sandy loam	7,500	23·8	315	6·9
Sacramento fine sandy loam	4,460	17·6	253	7·0

Strontium was determined by a General Electric model *XRD-5* X-ray spectrograph, and we are greatly indebted to Dr. L. D. Whittig for instructing us in the use of this instrument. The general procedure of Brandt and Lazar[2] was modified[3] to enable a working curve to be prepared without the use of chemically analysed samples. The soils were extracted[4] with ammonium acetate–ammonia at *p*H 9·0.

The results (Table 2) indicate five significant features. (1) Every legume species takes up more strontium than any grass—three to six times as much in many cases. (2) Within either the grasses or the legumes the variation in strontium content is not marked. (3) The amount of strontium taken up is directly related to the amount of calcium taken up. (4) There is no major discrimination between strontium and calcium by either grasses or legumes. (5) Neither the grasses nor the legumes can be ranked in the same order of strontium content for every soil, but *Bromus inermis* and *Trifolium subterraneum* are consistently highest in their respective classes. Of the legumes, *Lotus corniculatus* has the least strontium, while *Phleum pratense*, *Dactylis glomerata* and *Phalaris tuberosa* are the lowest-ranking grasses.

The results indicate that animals grazing mixed legume-grass herbage, where the legume amounts to a significant proportion of the forage, will ingest much more strontium-90 than animals grazing all-grass pastures on equally contaminated soils. It is apparent that if the strontium-90 level in milk should ever

Table 2. STRONTIUM AND CALCIUM CONTENT OF PASTURE SPECIES GROWN ON THREE DIFFERENT SOILS

Species \ Soil	Nord fine sandy loam				Yolo fine sandy loam				Sacramento fine sandy loam			
	Ca (μeq./gm.)	Sr (μeq./gm.)	Ca/Sr ratio	Sr/Ca in plant / Sr/Ca in soil extract	Ca (μeq./gm.)	Sr (μeq./gm.)	Ca/Sr ratio	Sr/Ca in plant / Sr/Ca in soil extract	Ca (μeq./gm.)	Sr (μeq./gm.)	Ca/Sr ratio	Sr/Ca in plant / Sr/Ca in soil extract
Grasses:												
Bromus inermis, Manchar	348	1·49	250	1·4	202	0·83	242	1·3	306	1·25	245	1·0
Lolium perenne, Oregon Commercial	366	1·31	279	1·1	145	0·74	195	1·6	258	0·89	289	0·9
Lolium perenne, *S.*101	390	1·19	328	1·0	165	0·71	231	1·3	249	0·89	279	0·9
Festuca elatior, Late Ötofte	352	1·19	328	1·1	136	0·65	207	1·5	240	0·89	269	0·9
Festuca arundinacea, Alta	306	1·07	286	1·1	141	0·71	197	1·5	202	0·89	226	1·1
Phleum pratense, American Commercial	235	1·01	233	1·4	112	0·59	188	1·3	131	0·59	220	1·1
Phalaris tuberosa, Harding grass	352	0·95	369	0·9	151	0·71	211	1·4	225	0·71	315	0·8
Dactylis glomerata, Potomac	235	0·93	251	1·3	141	0·65	216	1·4	198	0·77	256	1·0
Legumes:												
Trifolium subterraneum, Tallarook	1,500	4·70	319	1·0	1,050	3·99	263	1·2	1,130	4·22	268	0·9
Medicago sativa, Caliverde	995	3·81	261	1·2	680	2·44	279	1·1	812	2·65	306	0·8
Trifolium fragericum, Salinas	1,105	3·57	310	1·0	573	2·20	260	1·2	780	2·74	285	0·9
Trifolium repens, *S.*100	1,020	3·51	291	1·1	521	1·96	266	1·1	755	2·62	288	0·8
Trifolium repens, Dutch White	995	3·27	304	1·1	770	2·84	271	1·1	985	3·45	286	0·9
Trifolium pratense, Kenland	940	3·20	294	1·1	667	2·47	270	1·1	1,130	3·99	283	0·9
Trifolium repens, Ladino	976	3·09	319	1·0	770	2·74	281	1·1	1,050	3·57	294	0·9
Lotus corniculatus, Los banos	718	2·53	284	1·1	390	1·67	234	1·3	648	2·38	272	0·9

rise to a level which, maintained over a period of time, might give cause for concern, it would be desirable to restrict dairy cows to an all-grass diet. On current evidence this situation is unlikely to occur if the present level of atomic weapon testing is maintained, but in the future it might prove desirable to take this measure to reduce the strontium-90 level in milk, particularly that consumed by children. Such measures might first become necessary in regions of high rainfall, or where other local factors predispose a higher than average radiostrontium content of milk. Milk radiostrontium contents as high as 32 s.u. have been reported[5].

Where land that is relatively heavily contaminated with strontium-90 must be actively farmed, then bare fallow should be sown with an appropriate grass, no clover being included in the mixture. Established pastures should be heavily fertilized with nitrogen to encourage the growth of grass at the expense of clover.

This work was carried out in the Department of Agronomy, University of California, Davis, California.

P. B. Vose

Welsh Plant Breeding Station,
University College of Wales,
Aberystwyth.

H. V. Koontz

Department of Agronomy,
University of California,
Davis, California.

[1] Comar, C. L., Russell, R. S., and Wasserman, R. H., *Science*, **126**, 485 (1957).
[2] Brandt, C. S., and Lazar, V. A., *Agric. and Food Chem.*, **6**, 306 (1958).
[3] Vose, P. B., and Koontz, H. V. (unpublished information).
[4] Bowen, H. J. M., and Dymond, J. A., *J. Exp. Bot.*, **7**, 264 (1956).
[5] United Press Agency Report (New York, July 4, 1958).

Plants and Fall-out

It has been suggested that the gist of certain unpublished observations on the condition of certain plants in the area of the Marshall Islands affected by fall-out from the 1954 Bikini hydrogen bomb test should be made public in order to stimulate further investigation on the subject of cumulative effects of low-level radioactivity in plants. These observations have hitherto remained unpublished because of their preliminary and inconclusive nature.

In earlier fall-out surveys of 1954 and 1955, made by non-botanical observers, in the atolls of Rongelap, Ailinginae, Rongerik and Utirik no visible effects on plants were observed, although accumulation of radioactive materials in plant tissues was demonstrated. In the 1956 re-survey, under the auspices of the U.S. Naval Radiological Defense Laboratory, only casual observations were made on the condition of the plants, attention being concentrated on collection of material for analysis. Eight coral islet stations were examined which had been affected by different intensities of fall-out.

Later study of notes made on the vegetation on these islets showed abnormal or pathological conditions in certain plant species, increasing from islet to islet in the same order as the increase in fall-out intensity. Since the observations were not made systematically, nor with the possibility of visible pathological effects in mind, only the roughest of correlations is possible. Even so, the differences were striking enough to merit further attention.

On Gegen Islet, Rongelap Atoll, the station with the greatest fall-out exposure, of a total flora of 15 species, 13 showed conspicuous pathological or abnormal symptoms, ranging from plants dead or almost so to chlorosis, dead terminal twigs and mistletoe-like abnormal growths.

On Kabelle Islet, Rongelap Atoll, next most heavily exposed, 3 species showed some damage.

On all other stations at most one or two species showed effects, but on Eniwetak Islet, Rongerik Atoll, though only two species were involved, the symptoms, defoliation and die-back of twigs, were so conspicuous as to alter the appearance of the vegetation, causing a general grey colour in place of the normal green.

On Likiep Islet, Likiep Atoll, which had scarcely any measurable fall-out, no abnormal appearances of any sort were noticed.

The plants most widely affected were: *Suriana maritima* L., which was completely dead on Kabelle Islet and partially dead on other islets where it was observed; *Cordia subcordata* Lam., which on Gegen Islet was dead or almost so, and on Eniwetak Islet, where it is the most important tree in the vegetation, had the tips of almost all its twigs dead for 10–15 cm.; and *Pisonia grandis* R. Br., which was more or less leafless on the more affected islets and bore, on Gegen Islet, curious mistletoe- or witches-broom-like leafy branches of a dark green colour which have not been noted elsewhere on this species. The defoliation of the last species is not necessarily abnormal, as it happens during severe drought periods. Its association with the stations where fall-out was relatively heavy and without other evidence of drought makes it of interest.

In all, 43 species are known from Rongelap, Rongerik and Ailinginae, the three atolls heavily affected by fall-out. Only about half these plants grow on the four islets where the heaviest fall-out was recorded. Of these, 16 species were noted to show some abnormality. Two species of universal occurrence and great abundance, *Scaevola sericea* Vahl and *Tournefortia argentea* L. f., showed no abnormal appearance whatever.

It will be noted that the total flora of these islands is very small and made up of strand species, tolerant of high salt concentration and exposure to drying winds.

I am very familiar with the flora and vegetation of coral atolls, generally, and with the Marshall Islands, especially, so that the observations are of more significance than if I had less background.

It is not suggested that these observations are positive indication of cumulative effect of fall-out. There may be other unsuspected causes. However, the correlation between intensity of observed symptoms and intensity of recorded fall-out is at least suggestive, and would seem to justify further investigation. The extreme diversity in the reactions of different species growing under the same conditions to whatever caused these effects is of great interest. It suggests the advisability of using a wide variety of plants in any set of experiments designed to investigate the effects of radioactivity, as well as those of other environmental factors. It becomes harder and harder to generalize on the influence of any factor on plants.

F. R. Fosberg

Pacific Vegetation Project,
c/o National Research Council,
Washington 25, D.C.
Feb. 15.

Comparative Sensitivity to Radiation of Seeds from a Wild Plant grown on Uraniferous and Non-Uraniferous Soils

THE long-term effects of low-level chronic irradiation on living organisms have attracted increasing interest in connexion with the observed contamination from man-made radioactive sources over large areas of the Earth's surface. No experimental evidence exists that definite biological changes are induced in a large population under very low continuous exposure.

The assessment of sensitivity during the process of germination may provide a satisfactory approach, as seeds are available from plants of the same species that have—or have not—grown for ages in a radioactive environment, thus being continuously irradiated. We used seeds of *Andropogon filifolius* (Nees) Steud, a perennial grass[1], collected by one of us on neighbouring uraniferous (Kasompi) and non-uraniferous, but otherwise similarly mineralized, soils (Dikuluwe) in Katanga (Belgian Congo). The dose received by the sexual or somatic cells of these plants is impossible to calculate, mainly because the radioactivity of the superficial layers of the soil is extremely variable—from 10 to 50 times background.

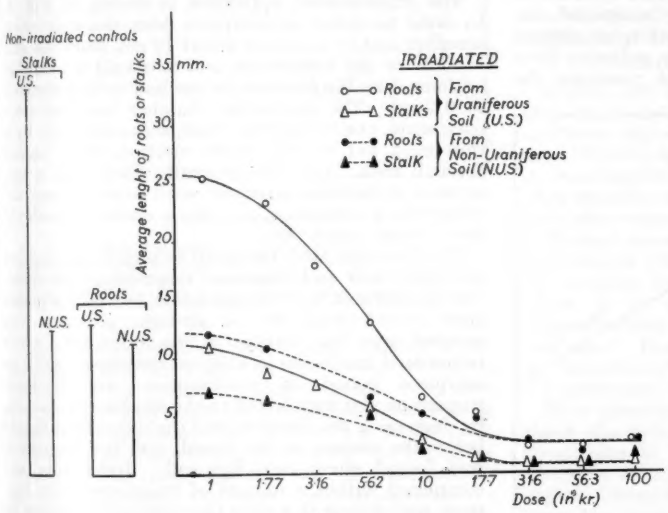


Fig. 1

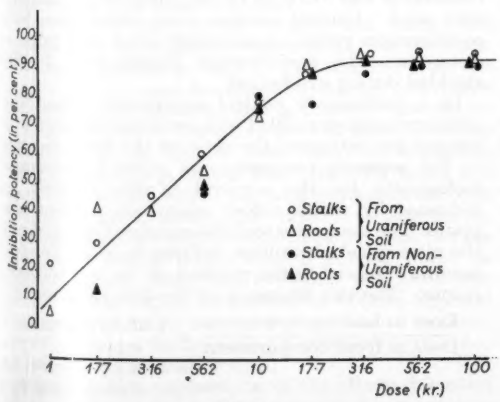


Fig. 2

Table 1

Dose (r)	Germination (per cent)	
	Uraniferous soil	Non-uraniferous soil
0	30·0	18·0
1,000	26·7	18·0
1,770	24·0	22·0
3,160	30·7	22·0
5,620	28·0	24·0
10,000	24·6	11·5
17,700	24·7	14·0
31,600	21·9	13·3
56,200	19·3	11·1
100,000	19·9	17·3

Groups of seeds (150) soaked for 12 hr. from both soils were irradiated simultaneously on moist filter paper at different doses with a Siemens 'Stabilivolt' (200 kV.; 20 m.amp.; no filter; source-target distance: 30 cm.; rate, 700 r./min.). All seeds were grown in a greenhouse on moist filter paper. Three criteria were investigated: germination, and maximal lengths of stalks and roots[2] at the time of death of the plantlets. The percentage of 'normal' germination and length of stalk and roots were estimated from un-irradiated control groups ($\pm$ 1·800 seeds). The results are shown in Table 1 and Fig. 1.

Seeds originating from uraniferous soil exhibit a higher percentage of germination, and plantlets develop stalks and roots of greater average length. Germination is not significantly inhibited by radiation in the range used. At any dose-level, the average length of roots and stalk is greater in plants from uraniferous soil. The potency of radiation in inhibiting growth of roots and stalks may be expressed as the percentage of shortening of initial length per roentgen. It decreased exponentially with dose and reached a plateau at the 20,000 *r.* dose-level; no significant difference was evident between either series (Fig. 2). However, the observed differences between percentages of germination and lengths of organs of plants from uraniferous soils and those from non-uraniferous soils lead to the conclusion that the two populations are different. The chronically irradiated population possesses higher vitality. Whether selection or adaptation or both are responsible is an open matter. In any event, enhancement of biological potentialities rather than degeneration has apparently resulted from long-continued low-level irradiation of *Andropogon filifolius*. This experiment is now being repeated with another species of *Andropogon*.

We thank the officers of the Union Minière du Haut Katanga and especially the geologists for their kind collaboration in collecting specimens.

D. J. MEWISSEN
J. DAMBLON
Z. M. BACQ
Laboratory of General Pathology and Therapy,
32 Bd. de la Constitution,
Liège.
April 17.

[1] Duvigneaud, P., *Bull. Soc. Roy. Bot. Belg.*, **90**, 127 (1958).
[2] Koernicke, M., *Ber. deut. Bot. Ges.*, **22**, 148 (1904).

Modification of the Oxygen Effect when Bacteria are given Large Pulses of Radiation

Many biological materials, including bacteria, have been shown to be more readily damaged when oxygen is available to the cell at the time of irradiation than when it is absent[1,2]. *Serratia marcescens* shows this enhanced sensitivity to radiation—the so-called 'oxygen effect'—almost to the full extent when a suspension of them is saturated with a gas mixture containing only 1 per cent oxygen and 99 per cent nitrogen, provided the radiation is given at normal dose-rates of about 1,000 rads/min. Typical inactivation curves for this organism using 1·5 MV. X-radiation from a pulsed linear accelerator are given in Fig. 1. Using the intense electron beam from the same linear accelerator, however, a dose of some 10–20 kilorads can be delivered in 2 microsec. The results quoted below indicate that when such large single pulses are used, the same bacteria saturated with the same oxygen–nitrogen mixture show the lower sensitivity corresponding to anaerobic irradiation. This is thought to be due to the fact that the first few kilorads of the electron pulse remove dissolved oxygen from the interior of the bacterium by radiation-induced reactions. During the remainder of the pulse the bacterium will thus be receiving radiation under essentially anærobic conditions. Even though dissolved molecular oxygen may not be entirely removed by the radiation from the extracellular liquid, this cannot penetrate the cell by diffusion in the 2 microsec. that the pulse lasts.

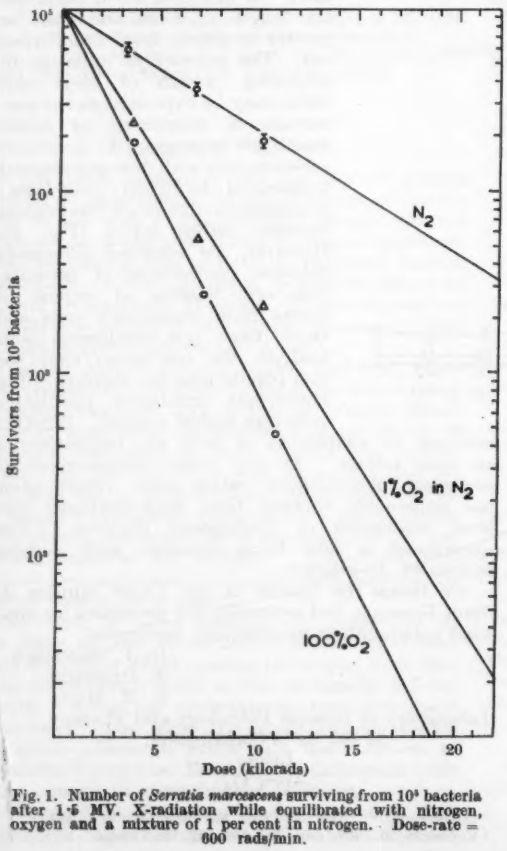


Fig. 1. Number of *Serratia marcescens* surviving from 10^5 bacteria after 1·5 MV. X-radiation while equilibrated with nitrogen, oxygen and a mixture of 1 per cent in nitrogen. Dose-rate = 600 rads/min.

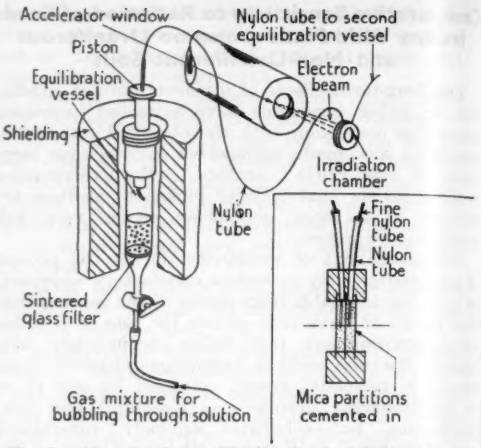


Fig. 2. Apparatus for equilibrating the bacterial suspensions and filling the irradiation chamber. Inset, an enlarged view of the irradiation chamber showing the two compartments

The experimental apparatus is shown in Fig. 2. In order to obtain an adequate dose, the irradiation chamber had to be placed about 10 cm. from the exit window of the accelerator, and to obtain a tolerably uniform dose distribution its size had to be restricted to 0·2 ml. The irradiation chamber had two compartments, one behind the other, separated only by a thin mica foil so that every electron pulse passed through both. One was generally filled with a suspension of bacteria saturated with nitrogen and the other with a suspension of bacteria saturated with the gas mixture under test.

The chambers were too small to permit internal gas bubbling, and each bacterial suspension was therefore equilibrated with the gas under test in a separate glass funnel closed by an air-tight plunger. Gas escaped from this through a fine nylon tube which terminated inside the irradiation chamber. After an adequate period of equilibration, the bacterial suspension was transferred to the irradiation chamber by depressing the plunger until the nylon tube dipped below the surface of the liquid, and the suspension was forced along into the cell. Irradiation was completed within a minute of transferring the bacteria, and during this time the removal of oxygen by respiration was found to be negligible at the bacterial titre used. Control samples were taken from both equilibration vessels immediately after each pulse of electrons. The equilibration vessels were heavily shielded during irradiation.

In a preliminary control experiment, when both compartments were filled with bacteria saturated with oxygen-free nitrogen, the ratio of the doses received in the separate compartments could be measured biologically by the survival of the bacteria, as indicated by visible colony counts on nutrient agar plates. The same ratio could be measured physically by the ultra-violet absorption induced in small 'Perspex' monitors[3] placed in the position of the cell compartments. The two estimates of the dose ratio were:

$$\frac{\text{Dose in back compartment}}{\text{Dose in front compartment}} = \begin{cases} 1{\cdot}36 \text{ by bacterial survival} \\ 1{\cdot}22 \text{ by ultra-violet absorption in 'Perspex'} \end{cases}$$

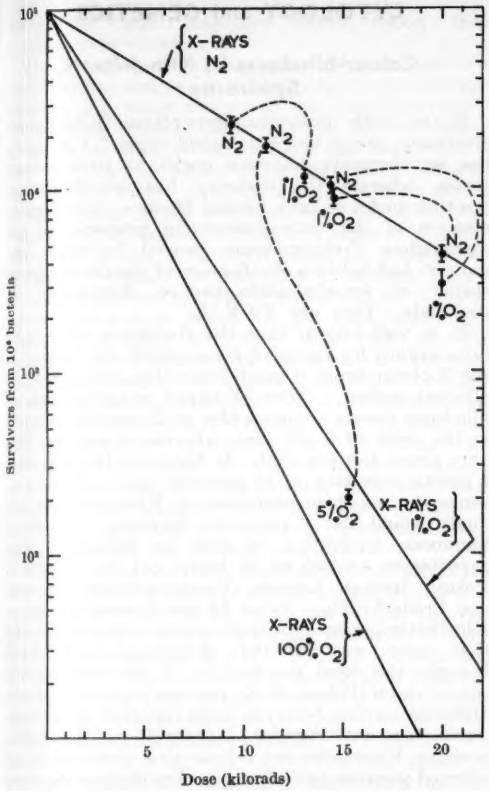


Fig. 3. The effect of single electron pulses on *Serratia marcescens*. The straight lines are X-ray survival curves taken from Fig. 1. The pairs of points joined by dotted lines are the survivors from the two compartments of the double cell, after a single pulse of electrons

The final conclusions were made largely independent of any differences in dose delivered to the two cells by doing each experiment twice—once with the reference suspension of nitrogenated bacteria in the front compartment and once with it in the back compartment. In plotting the results the biological estimate of the ratio, that is, 1·36, has been used as this was considered the more direct measurement; but the conclusions would be quite unaffected if the other value 1·22 had been used.

Fig. 3 is a plot of the observations made with pulsed doses in relation to the straight lines for lower dose-rates taken from Fig. 1. For each radiation the survival in the anaerobic compartment has been taken as a measure of the dose and is therefore plotted on the nitrogen line. The point derived from the other compartment is linked with the corresponding nitrogen point by a dotted line. Bacteria which had been equilibrated with 1 per cent oxygen in nitrogen showed approximately the anaerobic sensitivity, whereas bacteria equilibrated with 5 per cent oxygen in nitrogen showed nearly the full oxygen effect. In the latter case, there was evidently too much dissolved oxygen within the bacillus for it to be removed by the early part of the electron pulse and so most, or perhaps all, of the dose was delivered in the presence of sufficient dissolved oxygen.

An alternative explanation of the effects described above, based on radiation-chemical interaction between clusters of ions formed in adjacent electron-tracks in the single pulse, is unlikely to be correct. In order to approach the concentration of ions found in the core of an α-particle track an instantaneous dose of some 10^9 rads would be required.

Experiments with the pulsed irradiation are continuing to determine more exactly the shape of the survival curves for different initial concentrations of dissolved oxygen.

We are greatly indebted to Dr. L. H. Gray, who suggested this experiment, and has throughout given his advice and encouragement.

D. L. Dewey
J. W. Boag

Research Unit in Radiobiology,
British Empire Cancer Campaign,
Mount Vernon Hospital,
Northwood.
Feb. 24.

[1] Gray, L. H., "Organic Peroxides in Radiobiology", 104 (Masson et Cie, Paris, 1958).
[2] Gray, L. H., Chase, H. B., Deschner, E. E., Hunt, J. W., and Scott, O. C. A., Second United Nations Conference on Peaceful Uses of Atomic Energy Paper 15/*P*/293 (1958).
[3] Boag, J. W., Dolphin, G. W., and Rotblat, J., *Rad. Res.*, **9**, 589 (1958).

Action of Ultra-violet Light on Skin: Effect of the Histamine Liberator 48/80 and Methotrimeprazine

Rottier and Mullink[1] irradiated human skin with ultra-violet light at a place where the horny layer had been removed by repeatedly applying adhesive tape. The erythemal reaction practically disappeared at wave-lengths 250–260 mμ, but increased at 300 mμ. They therefore suggested that photolysable substances from the stratum corneum were responsible for the erythemal reaction caused by ultra-violet light of wave-length 250–260 mμ. The problem concerning the mediator substance in ultra-violet erythema still seems to be unsolved. Lewis and Zotterman's[2] suggestion that it should be histamine has not been confirmed[3]. Recently, much interest has been focused on 5-hydroxytryptamine as a possible mediator in the production of œdema.

It is therefore of interest to obtain information about the role taken by histamine and 5-hydroxytryptamine in the production of ultra-violet light erythema at the two wave-lengths mentioned above, both in intact skin and in skin where the superficial layer has been removed by tape. This has been done by treating the animals subjected to irradiation with 48/80, which liberates both histamine and 5-hydroxytryptamine, and with methotrimeprazine (7044 *RP*), an inhibitor of 5-hydroxytryptamine[4].

Experiments were performed on albino male rats (100–120 gm.) where the stratum corneum had been removed from one side of the shaved back by applying adhesive tape. For irradiation, the rats were fastened to boards and covered with black rubber sheets with three 16-mm. holes on each side of the spine. Radiation of wave-length 280–330 mμ was obtained from a water-cooled, high-pressure mercury arc (Philips *SP* 500 W.) provided with filter (2 mm. Jena *WG* 7 + 10 mm. $NiSO_4.6H_2O$, 600 gm./l.). For the wave-length 253·7 mμ, a low-pressure mercury arc was used. The doses, measured by chemical actinometry[5], varied from about 2×10^{16} to 5×10^{18} $h\nu$/cm.[2]. The degree of tissue damage was estimated from the leakage of intravenously injected Evans blue into the irradiated areas[5,6].

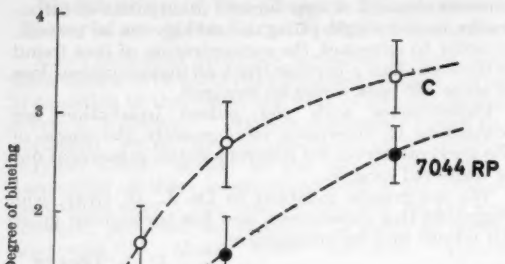


Fig. 1. Degree of blueing (tissue injury) versus dose for rats treated with 7044 *RP* after irradiation (●) and for control animals (○). The vertical lines indicate the standard deviation from the mean

Of the 60 animals used, one third were pre-treated intraperitoneally with compound 48/80 in increasing doses from 100 to 700γ for 7 days previous to irradiation. Another third were injected intravenously immediately after irradiation with 0·5 mgm. 7044 *RP*. The remainder were used as controls, and irradiated simultaneously with the other animals.

In all cases, and for both wave-lengths, removal of the corneum caused increased erythemal reactions at both 253·7 and 280–330 mμ. Thus for rats, the results at the wave-length 253·7 mμ are not in accordance with those found by Rottier and Mullink[1] for humans. To exclude the possibility that the difference is due to the different species used, experiments were also performed on rabbits and humans; in both cases removal of the corneum caused an increased reaction at the wave-length 253·7 mμ.

No observable change in the degree of blueing could be observed by depleting the rat-skin of its content of histamine and 5-hydroxytryptamine by use of compound 48/80.

On the other hand, the interesting observation was made that the ultra-violet light reaction in rats at 280–330 mμ could be decreased by injection of a 5-hydroxytryptamine inhibitor immediately after irradiation. This is evident from Fig. 1. These results, together with the observation[3] that a histamine inhibitor has no effect, indicate that the mediator substance in the ultra-violet reaction is not histamine, but it may be 5-hydroxytryptamine.

S. Claesson
G. Wettermark

Institute of Physical Chemistry,

L. Juhlin

Departments of Pharmacology and Dermatology,
University of Uppsala.
Feb. 7.

[1] Rottier, P. B., and Mullink, J. A. M., *Nature*, **170**, 574 (1952).
[2] Lewis, T., and Zotterman, Y., *Heart*, **13**, 203 (1926).
[3] Partington, M. W., *Clin. Sci.*, **13**, 425 (1954).
[4] Parrat, J. R., and West, G. B., *Brit. J. Pharmacol.*, **13**, 65 (1958).
[5] Claesson, S., Juhlin, L., and Wettermark, G., *Acta Dermato-Venereol.*, **38**, 123 (1958).
[6] Claesson, S., Juhlin, L., and Wettermark, G., *Nature*, **180**, 902 (1957).

CYTOLOGY and GENETICS

Colour-blindness in Klinefelter's Syndrome

Males with testicular hypoplasia (Klinefelter's syndrome) are of two cytological types. A minority has sex-chromatin-negative nuclei as have normal males, whereas the majority has sex-chromatin-positive nuclei as have normal females. The demonstration of the male-determining property of the mammalian *Y*-chromosome, proved for man[1] and mouse[2], had led to a clarification of the chromosomal status of sex-chromatin-positive Klinefelter individuals. They are *XXY* [1,2].

It is well known that the frequency of females homozygous for recessive genes which are located in the *X*-chromosome is much lower than of hemizygous affected males. Thus, *X*-linked red-green colour-blindness occurs among males of European ancestry in the order of 8 per cent, whereas in females it is only about 0·5 per cent. It has been thought that a genetic correlate of the presence of two *X*-chromosomes in sex-chromatin-positive Klinefelter individuals would be a very low frequency of colour-blindness, equivalent to that in females. This expectation seemed to be borne out by studies of Polani, Bishop, Lennox, Ferguson-Smith, Stewart and Prader[3]. They tested 55 sex-chromatin-positive Klinefelter patients for their colour vision and found that none was affected. Additional tests have brought the total number to 72, all with normal colour vision (Polani, R. E., personal communication). Different results, however, were reported by Nowakowski, Lenz and Parada[4]. Among 34 sex-chromatin-positive Klinefelter individuals they detected three affected persons, two of whom were deuteranomalous and one protanopic. It will be shown that the findings of the two groups of investigators are compatible with each other and that they may provide new insight into the behaviour and constitution of human chromosomes.

One of the deuteranomalous men had a deuteranomalous mother and a father with normal colour vision. Assuming *XXY* constitution of the propositus, he would be expected to be deuteranomalous if he originated from a non-disjunctional egg carrying two maternal *X*-chromosomes fertilized by a sperm carrying a *Y*-chromosome. The other deuteranomalous and the protanopic propositus both had normal fathers, and mothers who, on independent evidence, were heterozygous for an allele for defective colour vision. How can the defective colour vision of the presumably *XXY* propositi be explained? There is a possibility, of unknown strength, that they are manifesting heterozygotes[5]. But in *Drosophila* and other organisms the phenomenon of homozygous recessive offspring from heterozygous mothers mated to hemi- or homo-zygous dominant males is well known. It occurs in consequence of crossing-over and non-disjunction. If the chromosomes of the mother carry the alleles *A* and *a*, then at meiosis four chromatids, two carrying *A* and two *a*, will be present. If no crossing-over occurs between the kinetochore and the locus of *A*, non-disjunction at the first meiotic division will produce an *Aa* egg; but if within the region designated, certain types of cross-overs occur, an 'equational' non-disjunctional egg of the type *AA* or *aa* can be formed. This apparently had occurred in the eggs which gave rise to the two presumably homozygous Klinefelter

individuals with defective colour vision from heterozygous mothers.

The frequency of homozygosis due to crossing-over and non-disjunction is a function of the distance between the kinetochore and the locus concerned. With a map distance between these two so great as to result in free recombination, the maximum of 16·7 per cent of recessive homozygosis is obtained (leaving out minor complications). In other words, as the result of non-disjunction of the *X*-chromosomes in heterozygous females a maximum of about one-sixth of the *XXY* sons could be homozygous recessives. Since the frequency of women heterozygous for one or the other of the alleles for *X*-linked colour-blindness is about 15 per cent, maximally one-sixth of their Klinefelter sons from non-disjunction in the mothers, or maximally 2·5 per cent of all Klinefelters of this origin, could be colour-blind. In addition, approximately 0·5 per cent of Klinefelters would be colour-blind coming from homozygous affected mothers. A further source of colour-blind Klinefelter sons could be non-disjunction of the *X*- and *Y*-chromosomes in colour-blind men married to heterozygous or homozygous affected women. Approximately 0·5 per cent of Klinefelter individuals would be colour-blind due to this mechanism.

Depending on the relative proportion of individuals who owe their Klinefelter status to non-disjunction in the mother as compared to the father, the frequency of colour-blindness among them would vary between a maximum of 2·5 + 0·5 = 3 per cent and 0·5 per cent. The maximum expectation is statistically compatible with the observations of both groups of investigators, namely, 0 affected to 72 not affected, and 3 affected to 31 not affected. Combining the data results in 3 affected out of 106 individuals, or 2·8 per cent. The statistical uncertainty of this value is, of course, great.

An alternative hypothesis to the one proposed here would be that in man equational exceptions originate without crossing-over as the result of non-disjunction at the second meiotic division. If this were the only source of colour-blind Klinefelter males, they would constitute 7·5 per cent (from heterozygous mothers) plus 0·5 per cent (from homozygous mothers) of all propositi.

All three colour-blind individuals originated after non-disjunction in their mothers. Their frequency of 2·8 per cent suggests that most Klinefelter males with positive sex chromatin come from this source. Assuming that the first hypothesis proposed is correct, it suggests that the frequency of crossing-over among non-disjunctional eggs, leading to equational exceptions, is high. Furthermore, the high frequency of equational exceptions suggests that the locus for colour-blindness lies at a considerable distance from the kinetochore of the *X*-chromosome.

CURT STERN

Department of Zoology,
University of California,
Berkeley 4, California.

March 31.

[1] Jacobs, P. A., and Strong, J. A., *Nature*, **183**, 302 (1959). Ford, C. E., Polani, P. E., Briggs, J. H., and Bishop, P. M. F., *Nature*, **183**, 1030 (1959).

[2] Welshons, W. J., and Russell, L. B., *Proc. U.S. Nat. Acad. Sci.* (in the press). Russell, W. L., Russell, L. B., and Gower, J. S., *ibid.* (in the press).

[3] Polani, P. E., Bishop, P. M. F., Lennox, B., Ferguson-Smith, A. M., Stewart, J. S. S., and Prader, A., *Nature*, **182**, 1092 (1958).

[4] Nowakowski, H., Lenz, W., and Parada, J., *Klin. Wochenschr.*, **36**, 683 (1958); *Acta Endocrinologia*, **30**, 296 (1959).

[5] Walls, *A.M.A. Arch. Ophth.*, (in the press).

Two Types of Ribonucleoprotein in the Nucleolus of Mammalian Cells

NUCLEOLAR differentiation has been recognized since the time of Gustav Valentin. Agassiz[1] referred to a body within the nucleolus of the turtle egg as the "Valentinian vesicle". Montgomery[2] and Heidenhain[3] called this structure the nucleolinus. Recently, it has been suggested that the appearance of vesicular or solid nucleolini is due to the presence of a reticulum or thread called the nucleolonema[4]. None of the procedures that reveal nucleolonemata[4–6] has any cytochemical significance. Serra[7] suggested that the intranucleolar differentiation revealed by empirical procedures may be the result of physical rather than chemical inhomogeneity within the nucleolus. Chemical differences have, however, been found in the nucleoli of plant cells. Chayen *et al.*[8] demonstrated one or more inner zones of protein material and an outer zone that probably contained ribonucleoprotein. Further evidence of cytochemical differentiation in the nucleolus has been provided by a new stain for nucleoproteins[9].

Normal mouse and rat liver and pancreas, rat sympathetic ganglion, the Ehrlich and Krebs 2 ascites tumours, sarcoma 180 and a human epidermoid carcinoma were prepared by the toluidine blue–molybdate method to produce stage 1 of staining[9]. By this means, ribonucleoprotein of the parachromatin (nuclear sap) was coloured purple and that of the greater part of the nucleolus was stained green[9]. The nucleoli of many neurones, the parenchymal cells of liver and pancreas and neoplastic cells also contained minute unstained vacuoles and a variable amount of metachromatic ribonucleoprotein. The latter was most prominent in transplantable tumours, and especially in the Ehrlich tumour, where it was present in the nucleoli of every interphase cell in the form of minute granules, larger hollow spheres or clusters of hollow spheres (Fig. 1). Under the phase microscope numerous vacuoles were observed in the nucleoli of living cells (Fig. 2). Comparison of the nucleolus in the living cell with that in the stained preparations revealed that the metachromatic ribonucleoprotein was present in or around some, but not all, of the vacuoles (Figs. 1 and 2). This

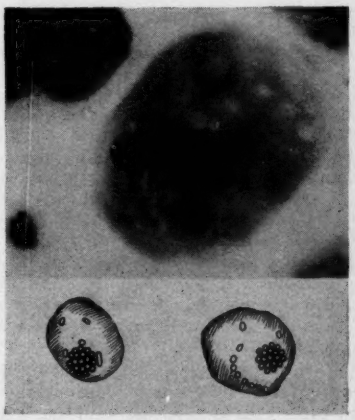


Fig. 1. Ehrlich ascites tumour cell stained by the toluidine blue–molybdate method (stage 1). Several minute vacuoles are visible in the green-staining outer zones of two prominent nucleoli. A cluster of metachromatic vesicles gives the appearance of a slightly eccentric internal body. (×1,400)

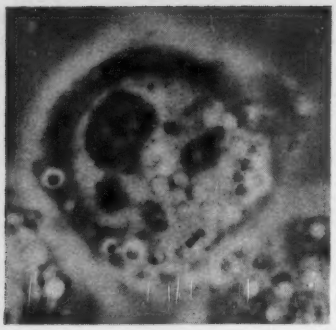


Fig. 2. Phase microscope photograph of an Ehrlich ascites tumour cell. A ring of vacuoles surrounds a dark structure in the nucleolus, in the centre of which a lighter vacuole is present. The central structure stains metachromatically by the toluidine blue–molybdate method. (× 1,400)

nucleolar ribonucleoprotein was coloured in exactly the same way as the nuclear parachromatin in all stages of staining by the toluidine blue–molybdate method and did not stain after pretreatment with ribonuclease or hot trichloracetic acid[7]; it may therefore be called the nucleolar parachromatin. In the Ehrlich ascites tumour the nucleolus often persisted throughout mitosis but was not incorporated into the daughter nucleus. In early prophase the nucleolar parachromatin was frequently at the rim of the nucleolus; during later prophase it was no longer detectable, whereas the vacuolated green-staining nucleolus was clearly visible in metaphase, anaphase and telophase. The nucleolar parachromatin was probably extruded from the nucleolus during prophase to contribute to the accumulation of granular parachromatin that took place at this time[9]. In the daughter nucleus the earliest sign of re-formation of the nucleolus was a metachromatic body which later acquired an outer coating of amorphous or vacuolated green-staining ribonucleoprotein.

The exact relationship of the two types of ribonucleoprotein in the nucleolus to the nucleolonemata and the nucleolini is not clear. In electron micrographs the nucleolonema was virtually absent in the Ehrlich tumour[6], in which the nucleolar parachromatin was most prominent. The absence of thread-like structures in the Ehrlich tumour was confirmed in electron micrographs kindly prepared by Mrs. Darlene Brindley in this laboratory. Similarly, the nucleolar parachromatin was least prominent in liver cells, where, according to Bernhard *et al.*[6], the nucleolus consists almost entirely of filaments. Although darker zones, resembling the nucleolar parachromatin, could be demonstrated around nucleolar vacuoles in rat sympathetic ganglion cells by Sotelo's method[4], the same technique did not reveal any internal structure, other than vacuoles, in the nucleoli of the Ehrlich tumour. The site and amount of nucleolar parachromatin is therefore unrelated to the ability of nucleoli to reduce iron or silver salts[4] or osmium tetroxide[5,6] to produce thread-like formations or nucleolonemata. Solid and hollow structures, resembling the nucleolini of the classical cytologists, were, however, demonstrable in Ehrlich cells stained with iron hæmatoxylin. Thus, the physico-chemical differences in ribonucleoprotein that determine differential staining by the toluidine blue–molybdate technique may also be responsible for differences in affinity for hæmatoxylin.

We wish to thank Mr. Roscoe H. Liles for valuable technical assistance and Mr. Walter C. Ashe for the drawing.

Robert Love
T. P. Bharadwaj
Laboratory of Pathology,
National Cancer Institute,
National Institutes of Health,
Public Health Service,
Department of Health, Education and Welfare,
Bethesda 14, Maryland.
March 2.

[1] Agassiz, L., "Embryology of the Turtle" (Little, Brown and Co., Boston, 1857).
[2] Montgomery, T. H., *J. Morph.*, **15**, 265 (1899).
[3] Heidenhain, M., "Plasma und Zelle" (G. Fischer, Jena, 1907).
[4] Estable, C., and Sotelo, J. R., "Fine Structure of Cells", 170 (Interscience Pub., Inc., New York, 1955).
[5] Borysko, E., and Bang, F. G., *Bull. Johns Hopkins Hosp.*, 468 (1951).
[6] Bernhard, W., Bauer, A., Gropp, A., Hagenau, F., and Oberling, Ch., *Exp. Cell Res.*, **9**, 88 (1955).
[7] Serra, J. A., *Nature*, **181**, 1544 (1958).
[8] Chayen, J., Davies, H. G., and Miles, U. J., *Proc. Roy. Soc.*, B, **141**, 190 (1953).
[9] Love, R., *Nature*, **180**, 1338 (1957).

Size Relationship of Oocytes and their Nuclei in Two Species of Ascidians

Nuclear intervention in cytoplasmic growth and differentiation has formed the subject of many studies during recent years. One significant result from these studies is the recognition of the major part played by the nucleolus in the developing egg[1–3]. A recent review by Vincent[4] has surveyed the problem.

The determination of size of the nucleus and nucleolus in different stages of growth of the oocyte is important in such a study. Very little work on volume relationships of the different cell structures during growth has been done, and, so far as I am aware, no information is available regarding ascidian oocytes. This communication presents the size relationships of the cell nucleus and nucleolus in two species of ascidians belonging to two different families, namely, *Ecteinascidia thurstoni* Herdman (Fam. Perophoridae : Enterogonia) and *Pyura* sp. (Fam. Pyuridae : Pleurogonia). *Ecteinascidia* is a viviparous form and *Pyura* is oviparous.

Fig. 1 presents the relationships of the diameter of the nucleolus, nucleus and the oocyte. In *Pyura* the oocyte grows to 150μ, while in *Ecteinascidia* it reaches 300μ in diameter. In both species, the diameter of the nucleus increases with increase in diameter of the oocyte. However, the increase in *E. thurstoni* is more striking than that in *Pyura*. In regard to the nucleolus in both species, there is an initial increase in size, which reaches the maximum —10μ diam.—when the oocyte has attained the diameter of 60μ in *Pyura* and 70μ in *Ecteinascidia*. There is no further increase in size of nucleolus after this and the curve levels off. The size relationships shown here for ascidian oocytes resemble those described for amphibian[5] and starfish[4] oocytes.

From these observations it would appear that, so far as can be judged by size, the nucleolus appears to be active only in the early stages of growth of the oocyte and not in the later stages. If the nucleolus plays any part at all in the synthesis of cytoplasmic proteins[1,3] it is clear that this activity is restricted to the early growth of the oocyte. Later contributions must come from some other source. Berrill[6] has posed the problem of the role of the follicle and 'test'

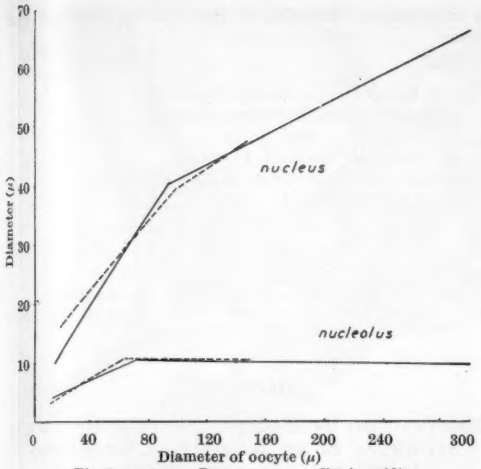


Fig. 1. ----, *Pyura*; ——, *Ecteinascidia*

cells in the growth of the ascidian oocyte. My observations on the later history of oocyte growth in both *Ecteinascidia* and *Pyura* lead me to believe that the activity of the nucleolus ceases, the follicle cells and 'test' cells take over, and, by their contributions, assist in the cytoplasmic synthesis. In these later stages, the outer half of the oocyte is filled with periodic acid–Schiff-positive substances, granules in *Ecteinascidia* and plaques in *Pyura*, and there is reason for believing that they come from the follicle and 'test' cells surrounding the oocyte. The periodic acid–Schiff reaction is indicative of the presence of protein carbohydrate complexes and it is highly probable that these are the predominant products of later synthesis.

A detailed study of the origin and nature of these substances is in progress and will be reported elsewhere.

I am indebted to Prof. B. R. Seshachar for his guidance and criticisms. Thanks are due to the National Institute of Sciences of India for awarding me a junior fellowship.

S. R. Venkatasubba Rao*

Department of Zoology,
Central College,
Bangalore, India. Feb. 24.

* Present address: Department of Cytogenetics, Cancer Institute (WIA), Gandhinagar, Madras 20, India.

[1] Caspersson, T., "Cell Growth and Cell Function" (1950).
[2] Brachet, J., "Biochemical Cytology" (1957).
[3] Vincent, W. S., *Int. Rev. Cytol.*, **4**, 269 (1955).
[4] Vincent, W. S., in "The Beginnings of Embryonic Developement", 1 (1957).
[5] Gall, J., Symp. Soc. Exp. Biol., **9**, 358 (1955).
[6] Berrill, N. J., "Tunicata" (1950).

Effect of Colchicine on the Utilization of Thymidine labelled with Tritium during Chromosomal Reproduction

Experiments[1] designed to verify the findings of Taylor, Woods and Hughes[2] on the incorporation of thymidine labelled with tritium in the deoxyribonucleic acid in chromosomes of growing root meristems of seedlings of *Vicia faba* have produced evidence that colchicine influences either the synthesis of the acid or its subsequent distribution among sister chromatids at metaphase. This evidence was derived from two types of experiment.

In the first, colchicine was not used and chromosomes with the sister chromatids both labelled were regularly found in metaphases and anaphases at 24 hr. after labelling and frequently also at 48 hr., that is, after approximately two cell cycles. Since after 48 hr. about 12 per cent of the metaphases contained some chromosomes labelled in both chromatids and others only in one, it was not of overriding importance to determine whether such nuclei were undergoing their first or second mitosis after the labelled thymidine was applied, though the indications were in favour of regarding them as second divisions.

In the second experiment, colchicine was used in the following way. Seedlings were grown in tap-water containing 0·05 per cent colchicine to which labelled thymidine was added 1 hr. later. This was replaced after 8 hr. by fresh tap-water with 0·05 per cent colchicine, and roots were fixed after 20 and 24 hr. In both samples autoradiographs showed some chromosomes labelled in only one chromatid already in the first mitosis after labelling. Closely similar results have since been obtained with concentrations of colchicine ranging from 0·025 to 0·1 per cent, treatment commencing 2 and 4 hr. before the application of labelled thymidine (Fig. 1).

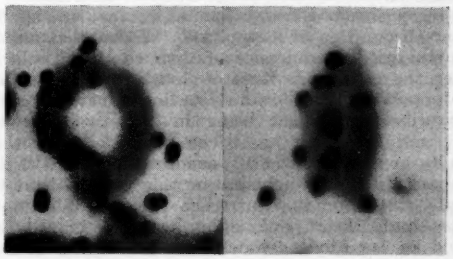


Fig. 1. Autoradiographs over *Vicia* chromosomes from *C*-metaphases at the first mitosis after labelling with ^{3}H-thymidine, showing labelling in one chromatid only. Left, from a root of a seedling grown for 2 hr. in 0·025 per cent colchicine in tap-water prior to treatment for 7 hr. with 0·025 per cent colchicine and ^{3}H-thymidine, followed by 0·025 per cent colchicine in tap-water for 13 hr. Right, after 4 hr. in 0·025 per cent colchicine; 0·025 per cent colchicine and ^{3}H-thymidine 7 hr.; 0·025 per cent colchicine 9 hr. Squash preparations stained in toluidine blue after autoradiography. (× 2,700)

These results seemed to contradict the findings of Taylor *et al.*, who reported that in their experiments all chromosomes were labelled in both chromatids at the first mitosis, while all were labelled in only one chromatid at the second mitosis. In a more recent publication, however, Taylor[3] reports two instances of chromosomes labelled in both chromatids in roots of *Bellevalia romano* in *C*-metaphases in the second division. The frequency of such chromosomes seems to be much lower than in our experiments on *V. faba* without colchicine. C. H. Ockey (personal communication), in agreement with our findings, has found chromosomes with sister chromatid labelling together with chromosomes labelled only in one chromatid in *V. faba* roots not treated with colchicine and fixed 40 hr. after labelling.

Woods and Schairer[4] grew seedlings of *V. faba* in nutrient solution containing labelled thymidine and 0·03 per cent colchicine for 12 hr. and compared them with plants grown in nutrient solution plus labelled thymidine for 8 hr. followed by nutrient solution with colchicine without labelled thymidine for 6 hr. Their experiment thus differs from our colchicine experiment in two important aspects: they omitted pretreatment for 1 hr. with colchicine

Table 1. Re-distribution of Newly Synthesized Deoxyribonucleic Acid
(Based on the experimental data of Woods and Schairer. 2 × 25 chromosomes each treatment)

Suggested ratio	1 : 1		1·5 : 1		1·75 : 1	
Treatment	χ^2	P	χ^2	P	χ^2	P
Control	43·61	0·72	23·44	>0·99	55·07	0·3
Colchicine	35·29	0·94	19·1	>0·99	45·91	0·5

The values for χ^2 for the ratios 1·5 : 1 and 1·75 : 1 were calculated on the assumption that the chromatid showing the higher number of grains represented the more heavily labelled one; the inverse probability of the more heavily labelled chromatid showing fewer grains was neglected. It is considered unlikely that the error thereby introduced should affect the conclusions.

to ensure that this agent was already active when labelled thymidine was added and the concentration of colchicine was lower than in our experiments. It should also be noted that the controls were kept in colchicine for 6 hr. after labelling and that the concentration of labelled thymidine was also different from ours. The extent to which these factors influence experimental results is under investigation.

Woods and Schairer counted grains in autoradiographs over 25 metaphase chromosomes in each sample and found by the chi-square test that their results were compatible with equal re-distribution of newly synthesized deoxyribonucleic acid among the sister chromatids at metaphase. We have examined the statistical significance of their results (Table 1). Since the chi-square tests obviously do not exclude the possibility of the re-distribution of newly formed deoxyribonucleic acid being in any ratio between 1 : 1 and 1·75 : 1 it has to be concluded that their results are not sufficiently accurate either to detect a change due to colchicine in this ratio within the limits stated or to contradict previous results by Plaut and Mazia[5], even if experiments on *V. faba* could be regarded as conclusive for *Crepis*. It is of interest to note that Plaut and Mazia calculated 2–3 sub-units per chromosome, which seems to agree well with the fit obtained for a factor of 1·5 : 1 in Table 1.

Woods and Schairer suggest that our observation of chromosomes with singly labelled chromatids at the first mitosis in roots of *V. faba* treated with colchicine is due to neglect of the influence on observations of absorption of β-particles in chromatids separated from the film by a layer of cytoplasm or other organic matter. The much-discussed short range of β-particles emitted by tritium was known to us when the autoradiographs were analysed and all chromosomes showing singly labelled chromatids were checked and doubtful ones rejected. Ockey (personal communication) and ourselves have found that the autoradiograph due to one labelled chromatid is still continuous where it is crossed by an overlying unlabelled chromatid. Although such differences in level can easily be seen by focusing, such chromosomes would certainly have been rejected by us. For these reasons, which inevitably introduce a certain element of subjectivity, and because of the foreseeable low accuracy, no grain counts were undertaken. It should be stressed that we never suggested that colchicine might affect the distribution of new deoxyribonucleic acid in all chromosomes; the claim was that chromosomes labelled in only one chromatid were found in significant numbers in metaphases at the first mitosis under the conditions of our experiment. In this sense the results of Woods and Schairer merely show that among the 50 chromosomes (that is, the complement of slightly more than four complete nuclei) which they accepted for grain counts no chromosome labelled in only one chromatid was found.

L. F. La Cour
S. R. Pelc

John Innes Horticultural Institution,
Bayfordbury, Hertford, Herts;
and
Medical Research Council
Biophysics Research Unit, King's College,
London, W.C.2. Feb. 27.

[1] La Cour, L. F., and Pelc, S. R., *Nature*, **182**, 506 (1958).
[2] Taylor, J. H., Woods, P. S., and Hughes, W. L., *Proc. U.S. Nat. Acad. Sci.*, **43**, 122 (1957).
[3] Taylor, J. H., *Genetics*, **43**, 515 (1958).
[4] Woods, P. S., and Schairer, M. U., *Nature*, **183**, 303 (1959).
[5] Plaut, W., and Mazia, D., *J. Biophys. Biochem. Cytol.*, **2**, 573 (1956); *Texas Repts., Biol. and Med.*, **15**, (1), 181 (1957).

BIOLOGY

Observations by Electron Microscopy on the Structure of an Acephaline Gregarine (*Apolocystis elongata*, Phillips and MacKinnon)

Apolocystis elongata lies in the seminal vesicles of the worm *Eisenia foetida* (Sav.). As we have seen by light microscopy, *Apolocystis* possesses a cuticle thickly covered by a pile of short hair-like processes each about 4μ long and 0·5μ thick. The processes do not show obvious signs of movement. The animal is a passive feeder. In our investigation by electron microscopy we have been particularly interested in determining the ultra-structural organization of the body-wall and hair-like processes.

Trophozoites of *Apolocystis* were fixed in 1 per cent osmium tetroxide solution buffered to *p*H 7·3[1], and embedded in a 3 : 1 mixture of *n*-butyl and *n*-methyl methacrylate. Ultra-thin sections were cut with a glass knife, mounted on to copper grids previously covered with a carbon film and examined with a Siemen's Elmiskop 1*a*.

The surface of the gregarine is covered with a thin, highly osmiophilic layer about 100 A. thick which covers a sheet-like array of vesicles, about one vesicle in depth. Each vesicle is about 200–300 A. in diameter and its limiting membrane is about 50 A. thick. The vesicles lie close to each other, so that in cross-section the body-wall appears to be delimited by a double membrane, the outer one corresponding to the membrane first described above and the inner one to the limiting membranes of the vesicles. Tangential sections through the body-wall show that the vesicles are arranged alternately. The general impression gained from such sections is that the body-wall is honeycombed with minute pores although, at present, we have no evidence that they are true pores in the sense that they open to the exterior and place the interior of the body in direct communication with the environment.

The electron microscope has revealed that the hair-like processes seen by light microscopy are simply finger-like evaginations of the body-wall (Fig. 1). The surface of each process has the same ultra-structural organization as the body-wall. Tangential sections through the surfaces of the 'hairs' reveal the honeycomb arrangement of the vesicles described above most clearly (Fig. 2). The interstices of the vesicles are filled with a weakly osmiophilic substance. Oblique sections through the long axis of the 'hairs' show what appear to be elongate fibres with cross-banding. Careful examination at high

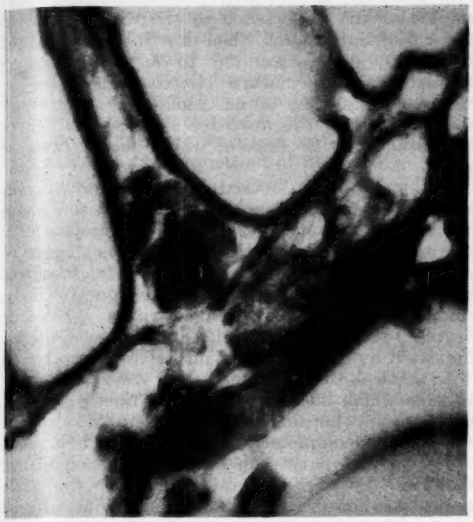


Fig. 1. Micrograph showing that the 'hairs' seen by light microscopy are finger-like evaginations of the body wall. At the base of one of the 'hairs' is a dense body of irregular outline and three smaller inclusions. Beneath the body wall is a membranous reticulum which in one region is concentrated to form an elongate body. (× 40,000)

resolution, however, shows that such 'fibres' are simply chains of vesicles, that is, the plane of section had passed through part of the wall. This interpretation is confirmed by the absence of fibres or any other osmiophilic content when the hair-like processes are seen in cross-section. In some micrographs we have observed a very dense osmiophilic granule of irregular outline and two or three smaller, and less-dense, spheroidal inclusions lying at the base of a 'hair' and projecting into it.

Functionally the hair-like processes seem to be a means of increasing the body-surface of the animal, thereby facilitating intake of nutriment and oxygen and elimination of waste products. The vesicles which lie in the body-wall probably serve essentially the same function.

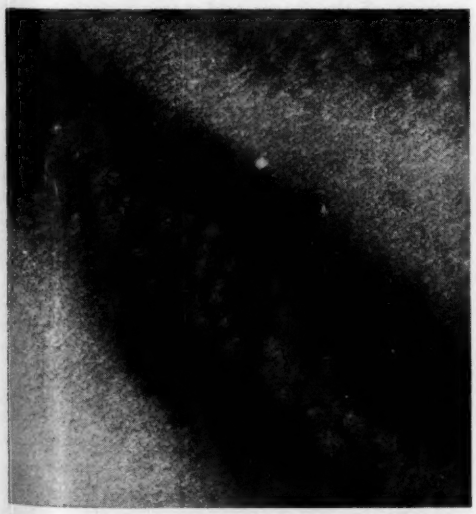


Fig. 2. Tangential section through the wall of a 'hair' to show the honeycomb arrangement of the small vesicles described in the text. (× 160,000)

Beneath the body-wall, underlying the vesicular system, is a complex network of membranes embedded in a homogeneous slightly osmiophilic substance (Fig. 1). The membranes and accompanying substance are generally separated from the vesicles by a clear (non-osmiophilic) zone of variable depth of the order of about 1μ. Each membrane is about 100 A. thick. Compared with inclusions seen in metazoan cells the network of membranes most closely resembles the endoplasmic recticulum[2]. Examination of light microscopical preparations of *Apolocystis* shows that the region occupied by the membranes and accompanying substance is strongly basophilic; the same has frequently been reported for regions containing much endoplasmic reticulum. However, in the latter case the basophilia is generally attributed to fine particles[2,3] which occur close to, or in contact with, membranes of the endoplasmic reticulum. In *Apolocystis* the basophilia is due either to the membranes or to the accompanying homogeneous substance, or to both.

In some regions the membranes and accompanying substance are concentrated in the form of spheroidal or elongate bodies which appear to be similar to mitochondria seen in micrographs of other tissue. Occasionally, many dense granules of irregular outline, of the same type as seen in the base of the 'hairs', lie enclosed by the meshes of the membranous network. Larger inclusions, spheroidal in shape and about 1μ in diameter, also occur within the meshes of the network.

Within the remainder of the animal occur many large vacuoles, each with a smooth limiting membrane about 50 A. thick. Examination of light microscopical preparations stained by Best's carmine method shows that such 'vacuoles' correspond to inclusions containing glycogen (glycogen grains). Between the 'vacuoles' lie extensions of the membranous system and inclusions referred to above. Occasionally, we have observed groups of membranes and small vacuoles (= dilations or paired membranes) which together resemble the Golgi apparatus seen in other Protozoa and in various Metazoa[4].

The nucleus is variable in position and consists of two main components, a prominent nucleolus and a peripheral zone. The nucleolus is very dense in appearance being composed of numerous small, highly osmiophilic granules. Within the nucleolus are several large vacuoles. The peripheral zone of the nucleus consists of strings of osmiophilic material lying within a 'clear' matrix. The nuclear contents are bounded by a double membrane, a feature which appears to be a characteristic of nuclei throughout the animal kingdom.

We are grateful to the Wellcome Trust for a grant to purchase an electron microscope and to St. Bartholomew's Hospital and Medical College, and the University of Hull, for further financial help and facilities.

D. Lacy
Department of Zoology and Comparative Anatomy,
St. Bartholomew's Hospital Medical College,
University of London.

H. B. Miles
Department of Zoology, University, Hull.

[1] Palade, G. E., *J. Exp. Med.*, **95**, 285 (1952).
[2] Porter, K. R., *J. Histochem. and Cytochem.*, **2**, 346 (1954).
[3] Palade, G. E., *J. App. Phys.*, **24**, 1419 (1953).
[4] Grassé, P. P., Proc. Stockholm Conf. on Electron Microscopy, 146 (Almquist and Wiksell, Stockholm, 1956).

Pogonophora in the Hardangerfjord, Western Norway

Until recently, Pogonophora were known only from the north-western Pacific and from Indonesia. All species being found in the deep sea such a limited distribution seemed unlikely, the more so as the tubes of the animals are hair-like and easily overlooked. In fact, new investigations and revision of old collections have proved that the Pogonophora are widely distributed and also occur in relatively shallow water. Thus one species is now known from the Polar Sea, and Kirkegaard found Pogonophora in the *Galathea* collections from Indonesia[1] and from the Gulf of Panama[2]. Jägersten[3] described *Siboglinum ekmani* from old collections from about 500 m. in the Skagerrak, and he also described the larva of another species from only 180 m. depth in the same area[4]. Kirkegaard[5] re-investigated the deepest parts of the Skagerrak and found more material of *S. ekmani*, and Southward[6–8] has obtained several species from the continental slope south-west of England and west of Ireland.

As the fauna of the Norwegian Deep in the Skagerrak comprises many typical deep-sea animals which also live in the deep Norwegian fjords, Pogonophora could be suspected to occur in our rich collections from the Hardangerfjord. Several samples of undetermined narrow tubes were re-investigated. Between them were found many still more slender, red-brown tubes up to 17·5 cm. long and strongly resembling those of *Siboglinum ekmani*. New dredgings in different parts of the Hardangerfjord have proved this species to be fairly abundant in depths between 396 and 840 m.

The material has been sent to Prof. G. Jägersten, who has confirmed that part of it is very similar to *S. ekmani*, whereas one animal probably belongs to another species. Thus at least two species of Pogonophora seem to occur in the Hardangerfjord.

Hans Brattström

Biological Station,
Espegrend, Norway.
March 6.

[1] Kirkegaard, J. B., Galathea Report, **2**, 79 (1956).
[2] Kirkegaard, J. B., Galathea Report, **2**, 183 (1956).
[3] Jägersten, G., *Zoologiska Bidrag fran Uppsala*, **31**, 211 (1956).
[4] Jägersten, G., *Zoologiska Bidrag fran Uppsala*, **32**, 67 (1957).
[5] Kirkegaard, J. B., *Nature*, **181**, 1086 (1958).
[6] Southward, A. J., and Southward, E. C., *Nature*, **181**, 1607 (1958).
[7] Southward, A. J., *Nature*, **182**, 272 (1958).
[8] Southward, E. C., and Southward, A. J., *J. Mar. Biol. Assoc. U.K.*, **37**, 627 (1958).

Utilization of Nutritive Spheres in the *Proteus* Group of *Amoebae*

In all members of the *proteus* group of *Amoebae*[1] there occur spherules of a pale blue colour to which the name 'excretion spheres' was given by early writers. This designation was changed to "nutritive spheres" by Hayes[2] in 1924. The reaction of these bodies to various reagents has been described in an appendix by Hayes to Taylor's paper entitled "*A. proteus*, some New Observations on its Nucleus Life History and Culture", and further information about them was later published by Taylor[3].

The diameter of these bodies is proportional to the age of the *Amoeba*. A quick examination of the nutritive spheres enables the observer to distinguish a newly metamorphosed *Amoeba* from an adolescent, mature or senile creature. It is necessary here to emphasize the importance to the reader of stating the age of the *Amoeba* when describing its reactions, behaviour, etc. Reaction to a certain stimulus characteristic of mature *Amoebae* may be very different from that of an adolescent. To repeat: the diameter of the nutritive spheres is a criterion of the age of the *Amoeba*, which should always be given.

In my paper[2] I used a hæmatoxylin which stained the nutritive spheres purple. In this account it was shown that during the differentiation of the spores in the adult *Amoeba* the nutritive spheres were gradually used up, so that those *Amoebae* which were full of spores could be picked out by the extreme scarcity of coloured nutritive spheres, the degree to which sporulation had proceeded being indicated by the number of nutritive spheres present.

In sections through sporulating *Amoebae*, nutritive spheres which had begun to yield up their contents looked like collapsing balls, every stage from a fully inflated to a completely collapsed sphere being found.

Recently, I have seen this phenomenon in living material. Under natural conditions the *proteus* group[1] are mature and senile in the winter months. If these mature specimens, easily identified by their large nutritive spheres (7μ), be placed in a Petri dish and kept under observation, the gradual loss of contents of the nutritive spheres, as sporulation proceeds, can be witnessed; deformed spheres of every kind, that is to say, spheres having given up varying volumes of their contents, can be picked out.

Monica Taylor

Convent of Notre Dame,
Glasgow, W.2.
Feb. 11.

[1] Jepps, M. W., "The Protozoa, *Sarcodina*" (Oliver and Boyd, Edinburgh, 1956).
[2] Taylor, M., *Quart. J. Micr. Sci.*, Appendix, 69 (1924).
[3] Taylor, M., *Nature*, **143**, 685 (1939).

Second Flowering in *Oryza sativa* (var. *indica*)

In India rice is normally harvested when ripe by cutting off the leaves and stems near ground-level. Shortly before this operation the field is allowed to dry. There is very little regeneration, though it is well known that rice can be propagated vegetatively by 'ratooning'. In 1958 three small plots of 5·2 square metres each at Giridih in Bihar were planted with the *aus* (rapidly maturing) variety *CH*-10 in July. Owing to the late monsoon the crop was poor. Instead of drying and cutting as usual, the plots were not allowed to dry, and the ears were plucked by hand on October 10. The plots produced a second crop which was harvested on November 23 by the usual method. Table 1 shows the yield of paddy for two harvests of the three plots.

The total yield was thus increased by 117 per cent, that is to say, more than doubled. The second crop was harvested at the same time as the *aman* (slowly maturing) rice in the neighbouring plots.

Table 1. Yield of Paddy (dry weight, gm.) for Three Plots each 5·2 square metres in Area for the First and the Second Crop

Yield \ Plots	1	2	3	Total
First crop	2·3	605·4	673·0	1,280·7
Second crop	660·2	650·8	187·8	1,498·8

Regeneration was found to occur in two ways—through the formation of new tillers at the base and new branches at some nodes of the stems of the harvested plants. Both the new tillers and the new branches flowered and thus produced the second crop. Experiments are being made this year on a large scale with numerous varieties to determine the economic value of this method of harvesting.

SUBODH KUMAR ROY

Biometric Research Unit,
Indian Statistical Institute,
Calcutta 35.
March 26.

Control of Leaf Senescence by Auxins

THE considerable knowledge which has accumulated over the past thirty years suggests that differentiation, growth and senescence of plant cells is closely linked to the auxin status of the tissue. Investigations by Shoji, Addicott and Swets[1] have shown that differentiating and enlarging tissues of the green leaves of Black Valentine bean contain a relatively high concentration of an extractable auxin. These levels decrease as the tissues mature and at senescence only small amounts of the auxin are detectable.

Although mature parenchymatous tissues can often be stimulated experimentally into renewed growth and cell division by supplying an auxin, the changes which take place in highly differentiated cells, such as the mature mesophyll of the leaf, are normally irreversible and their senescence and death are inevitable.

Recent work has now shown that the ageing processes in leaves can be arrested by treatment with certain chemicals, and in this way particular physiological states have been maintained for considerable periods of time. For example, Richmond and Lang[2] have shown that chlorophyll and protein breakdown and the associated loss of green colour in detached leaves of *Xanthium* can be retarded by treatment with kinetin, with a resulting extension of the life of the leaf. Person, Samborski and Forsyth[3] have found similar effects following the treatment of detached wheat leaves with benzimidazole. Brian, Petty and Richmond[4] have reported that both the natural autumnal yellowing and the subsequent leaf fall of a number of temperate deciduous trees have been delayed by spraying gibberellic acid on to the leafy branches.

From the work carried out in this Unit over a number of years it is clear that the ability to arrest physiological changes associated with leaf senescence is not a property only of kinetin, benzimidazole or the gibberellins. Synthetic auxins of the chlorophenoxyacetic acid group must also be considered. It has already been shown[5] that the natural and continuous fall in pectin methylesterase activity which occurs in the abscission zones of growing petioles of *Phaseolus vulgaris* can be prevented by treatment with 2,4-dichloro- or 2,4,5-trichloro-phenoxyacetic acids (2,4-D or 2,4,5-T) and that under these conditions, abscission and senescence of the petiolar tissues is prevented. The application of 2,4,5-T to the leaf blades of certain tropical trees has produced differential senescence within the treated leaves[6]; with suitable doses the areas immediately below the applied droplets remain permanently green and healthy while the surrounding areas become progressively yellow and senescent.

It has now been clearly demonstrated that the application of a suitable formulation of either 2,4-D or 2,4,5-T will prevent normal senescence in both attached and detached leaves of a number of temperate deciduous species. Most of the experiments have been done with *Prunus serrulata senriko* and the following procedure was adopted. During the months of October and November a drop of ethanol solution of the butyl ester of 2,4-D or 2,4,5-T was applied to the lamina on either side of the main vein to give a total dose per leaf ranging from 10 to 100 μgm. After this treatment an apparently normal yellowing and senescence occurs in the untreated areas, but in the cells of the tissues directly below the applied drop the plastids retain their chlorophyll so that, at the time of normal leaf-fall at the end of November, each treated area remains as an isolated green spot about 1 cm. in diameter surrounded by yellow tissue. With detached leaves similarly treated and kept in high humidity in the light, the untreated parts of the blade and the control leaves become entirely yellow within twelve days, while the islands of 2,4-D-treated tissue remain green until general autolysis starts some 10–20 days later.

If the leaves are treated with a radioactive butyl ester of 2,4-D, labelled with carbon-14 in the carboxyl group, the autoradiographs of the whole leaf made at various intervals after the application show that the majority of the radioactivity is initially concentrated immediately below the treated areas with only traces of activity over the remainder of the blade. This pattern of distribution remains unaltered in detached leaves, but a week after attached leaves have been treated no radioactivity can be detected in the untreated portions of the blade; activity is then confined to the areas below the drops and eventually corresponds to the areas of tissue which remain green.

The colour of the islands of green cells is not due to an immobilization of chlorophyll in an inactive form. When the leaves treated with 2,4-D are illuminated and supplied with carbon-14 dioxide a ready fixation of carbon-14 occurs in the green areas beneath the applied 2,4-D, while fixation in the surrounding yellowing tissue is negligible and of the same order of magnitude as that in a yellowing control leaf.

Respiratory studies on disks of leaf tissue have shown that the treated areas which remain green retain a relatively high oxygen uptake which equals that of an untreated green leaf, while oxygen uptake in the surrounding yellowing tissue falls progressively for many days after the application of 2,4-D and follows that in a yellowing control leaf.

Preliminary analyses of the nitrogen fractions in the treated leaves indicate that the level of alcohol-insoluble nitrogen remains higher in the green 2,4-D-treated areas than in the surrounding yellowing tissue, suggesting that there may be an effect on protein conservation similar to that described for kinetin and benzimidazole.

The evidence for the maintenance of chlorophyll and of photosynthetic activity in leaf tissue following the application of high doses of 2,4-D is of particular interest, since previous reports by Wedding, Erickson and Brannaman[7] and Akers and Fang[8] have described depressions in photosynthesis following treatment with synthetic growth-regulators. Maciejewska-Potapczyk[9] records an actual reduction of 25–40 per cent in the chlorophyll content of leaves four days after treatment with 2,4-D, and Moreland, Hill and Hilton[10] have recently found that certain phenoxyacetic acids, including 2,4,5-T, have an inhibitory effect

on the photochemical Hill reaction in isolated chloroplasts.

The responses which occur in both plant growth and cellular metabolism following applications of auxins are usually considered as a departure from the control pattern. The results presented here show that, under certain conditions, 2,4-D and 2,4,5-T tend to maintain unchanged particular physiological states within the cells and thereby delay the normal changes which take place during senescence. These results thus provide a further example of the control of physiological ageing by auxins.

I am indebted to Prof. W. O. James, Dr. Mary Halloway, Dr. D. C. Smith, Mr. R. G. Powell and Dr. C. C. McCready for their co-operation in this work, and I wish to thank Prof. G. E. Blackman for his constant interest and encouragement. Further details will be published elsewhere.

Daphne J. Osborne

Agricultural Research Council Unit of Experimental Agronomy, Department of Agriculture, University of Oxford.

Feb. 19.

[1] Shoji, K., Addicott, F. T., and Swets, W. A., *Plant Physiol.*, **26**, 189 (1951).
[2] Richmond, A. E., and Lang, A., *Science*, **125**, 650 (1957).
[3] Person, C., Samborski, D. J., and Forsyth, F. R., *Nature*, **180**, 1294 (1957).
[4] Brian, P. W., Petty, J. H. P., and Richmond, P. T., *Nature*, **183**, 58 (1959).
[5] Osborne, D. J., *J. Exp. Bot.*, **6**, 446 (1958).
[6] Osborne, D. J., *Trop. Agric.*, **35**, 145 (1958).
[7] Wedding, R. T., Erickson, L. C., and Brannaman, B. L., *Plant Physiol.*, **29**, 64 (1954).
[8] Akers, T. J., and Fang, S. C., *Plant Physiol.*, **31**, 34 (1956).
[9] Maciejewska-Potapczyk, W., *Bull. Soc. Sci. et Lettres Lodz Classe III*, **4**, 12 (1953).
[10] Moreland, D. E., Hill, K. L., and Hilton, J. L., Abstr. Weed Soc. Amer., **40** (1958).

A New Nitrogen-fixing Micro-organism producing a Red Pigment

While isolating strains of *Rhizobium lupini* from the nodules of *Lupinus luteus* grown on the acid sandy soil of the Nyirség area in north-eastern Hungary, I succeeded in isolating, on a *Rhizobium* medium, two red micro-organisms. These have been labelled *Pr* and *E*.3. They came from places several miles apart. The plants from which the *Pr* and *E*.3 strains derived were in a state of fluorescence and conspicuously larger than the surrounding lupins.

The new micro-organisms are spherical or ovoid cells, 4–6μ in diameter. During their growth, 2–4 spherical, strongly refringent granules appear in the cells. A daughter cell becomes detached without a sterigma developing. In aged cultures large spherical cells with diameters between 8 and 9μ can be seen in which, besides the above-mentioned granules, 5–10 additional similarly shaped but smaller granules develop.

The new micro-organisms form a slowly migrating smooth colony on agar slants, with a strong adhesion to the nutrient medium.

The cell-wall gives a good response to most of the usual stains. Carmine acetic acid stains one point of the cell-wall.

Glucose, maltose, saccharose, lactose, mannitol and salicin are decomposed by the new strains with an intensive production of acids, but without the development of gases. They produce urease and do not liquefy gelatine. They produce no hydrogen sulphide and do not reduce nitrates to nitrites. They can utilize ethanol as their only source of carbon. Placed on a bean extract containing a 3 or 6 per cent solution of ethanol they will proliferate intensively. A temperature of 16° C. ensures vigorous growth, the optimum temperature being 25–28° C. Washed off the culture medium and exposed to ultra-violet rays they give a blue fluorescence. Diffused light promotes proliferation and pigment production. They are resistant to streptomycin, which does not impair their pigment production. Low concentrations of ammonium chloride, ammonium sulphate, potassium nitrate and peptone promote growth and the development of red pigment. The *p*H of culture media adjusted to 7 is reduced by them to 5·2–4·2 within 48 hr. They grow satisfactorily and continue to produce red pigment even in media containing 0·3 per cent citric acid, with a *p*H less than 3. Placed in stab cultures and examined otherwise they were found to be obligate aerobes.

Both strains develop satisfactorily on yeast, bouillon and bean agar, and fix atmospheric nitrogen without symbiosis while producing red pigment. To be able to do so the presence of a nitrogen-source in the nutrient medium is indispensable.

The properties described justify the claim that two varieties of a new kind of *Saccharomyces* have been discovered: the only difference between them is that the red pigment produced by strain *Pr* is brighter than that produced by *E*.3. No taxonomical allocation has yet been made.

The increase in nitrogen per unit of glucose consumed has been studied in numerous experiments on a bean-soup medium which in some cases had been aerated. Results were invariably positive. The significant experiments, which were repeated several times, showed the nitrogen increase to be 2·4–5·7 mgm. nitrogen/gm. glucose. Culture flasks through which air had been passed gave the higher value.

The results obtained from four different treatments (each repeated three times) are given in Fig. 1.

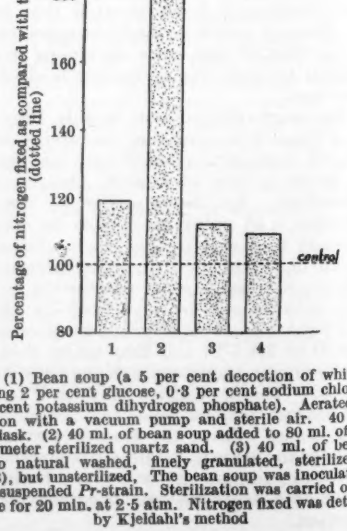


Fig. 1. (1) Bean soup (a 5 per cent decoction of white beans containing 2 per cent glucose, 0·3 per cent sodium chloride and 0·2 per cent potassium dihydrogen phosphate). Aerated during cultivation with a vacuum pump and sterile air. 40 ml. per culture flask. (2) 40 ml. of bean soup added to 80 ml. of 0·3–0·8 mm. diameter sterilized quartz sand. (3) 40 ml. of bean soup added to natural washed, finely granulated, sterilized sand. (4) As (3), but unsterilized. The bean soup was inoculated with 1 ml. of suspended *Pr*-strain. Sterilization was carried out in an autoclave for 20 min. at 2·5 atm. Nitrogen fixed was determined by Kjeldahl's method

That the value obtained in (4) is inferior to that in (3) is presumably due to the presence of antagonistic micro-organisms in the unsterilized sand as, otherwise, there was no difference between the two soils.

There was a correlation between the degree of nitrogen fixation and the red colour. This suggested that the red pigment may have a role in fixing atmospheric nitrogen similar to that of leghæmoglobin, which occurs in the nodules of leguminous plants and is known to be involved in symbiotic nitrogen fixation. The determination of a relationship between leghæmoglobin and the red pigment would be very useful in elucidating the chemical mechanism of atmospheric nitrogen-fixation.

Investigations with this end in view have been started in the Institute for Organic Chemistry at Szeged, and experiments performed under the supervision of G. Fodor by B. Matkovics produced the following results: (1) The red colour of the pigment produced by strain *Pr* is due to a hæmoprotein related to hæmoglobin as shown by the ultra-violet spectroscopic data of J. Hires; (2) a porphyrin ester from leghæmoglobin has been prepared[1].

Investigations on the exact structure of the pigment from strain *Pr* are being carried out by G. Fodor and co-workers in the Stereochemical Laboratory of the Academy, Budapest.

GY. NÉMETH

Agricultural Experimental Station,
Nyirtelek, Hungary. Feb. 23.

[1] *Acta Phys. Chem., Szeged*, **4**, 69 (1958).

Preparation of Autoradiograms for Electron Microscopy

FREQUENTLY when examining autoradiograms of biological specimens there is a need for resolution such as can be obtained with the electron microscope. A technique for preparing autoradiograms for electron microscopy has been reported by Liquier-Milward[1]. The method described here has the advantage of being somewhat simpler in that nuclear emulsion is applied directly to the specimen grids, and the method is suitable for use with ultra-thin sections. Although only results obtained with yeasts and the alpha-emitter polonium-210 are described, other isotopes and biological specimens can be used.

Yeast cells, previously suspended in a polonium-210 solution, were fixed with 2 per cent osmium tetroxide containing 0·8 per cent sodium chloride[2]. The fixed cells were dehydrated in a graduated series of ethanol concentrations and embedded in methacrylate. Good results were obtained with a mixture of four parts N-butyl methacrylate and one part methyl methacrylate[3]. Ultra-thin sections were cut with a Porter–Blum microtome, transferred to carbon-coated specimen grids, and allowed to dry thoroughly. All subsequent steps were carried out under light filtered through a Wratten series *OA* filter. Alpha-sensitive Eastman Kodak *NTA* emulsion in gel form (obtained through the courtesy of the Eastman Kodak Co., Rochester, N.Y.) was heated at 45° C. until fluid. A drop of the liquid emulsion, either full strength or diluted 1 : 1 with distilled water, was transferred to a clean glass slide. Specimen grids were coated by touching them to the drop of emulsion. Occasionally the yeast sections floated off when contact was made with the emulsion. This was due to insufficient drying of the grids before emulsion was applied. After coating, the grids were placed in a light-tight box containing a small vial of anhydrous calcium sulphate and exposed for 3–4 weeks at 4° C. The autoradiograms were developed for 15 min. in Eastman Kodak *D*-19 developer, fixed in acid fixer for 5 min., washed thoroughly in running tap water, and finally rinsed in distilled water. After drying they were examined with an *RCA EMU-2C* electron microscope.

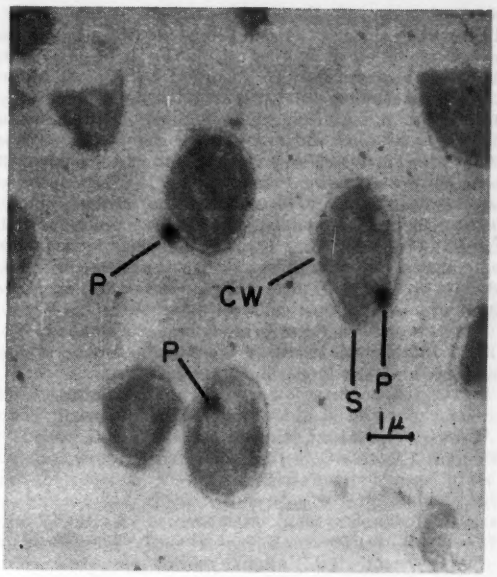


Fig. 1. Electron micrograph of autoradiogram showing distribution of polonium-210 on yeast cells. *P*, Polonium track; *CW*, cell wall; *S*, bud scar

Typical results are shown in Figs. 1 and 2. It is apparent that the emulsion obscured some cellular detail; however, in spite of this, good autoradiographic resolution of the location of polonium-210 in the cells was obtained. There was no evidence that

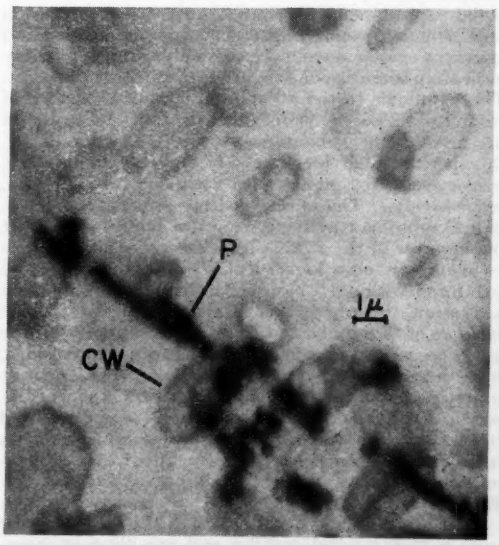


Fig. 2. Electron micrograph of an alpha track from polonium-210 on yeast cells. Key as in Fig. 1

sectioning caused displacement of the radioactivity. Due to the thinness of the film, only those particles which travelled at angles estimated to be less than 1° from horizontal were recorded as typical alpha tracks. Actually, this was an advantage since long tracks did not give the autoradiographic resolution that shorter ones did. A comparison of the figures illustrates this. In Fig. 1 the location of polonium-210 is much better defined than in Fig. 2. Radioactivity was almost entirely located on the surface of the cells, although a few were found to have polonium-210 in the cytoplasm (Fig. 1). This suggests that yeasts are nearly impermeable to this element. Further details on the distribution of polonium-210 and other heavy metals in yeast cells will be reported elsewhere.

This work was performed under Contract No. *W*-31-109-Eng-52 between the Atomic Energy Commission and the General Electric Co.

R. T. O'Brien
L. A. George, II.

Biology Operation, Hanford Laboratories, General Electric Co., Richland, Washington.

Feb. 27.

[1] Liquier-Milward, J., *Nature*, **177**, 619 (1956).
[2] Chapman, G. B., and Hillier, J., *J. Bact.*, **66**, 362 (1953).
[3] Agar, H. D., and Douglas, H. C., *J. Bact.*, **70**, 427 (1955).

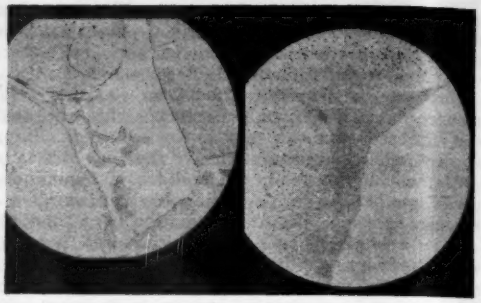
PHYSIOLOGY and BIOCHEMISTRY

Use of 'Tremorine' for screening Anti-Parkinsonian Drugs

'Tremorine' (1 : 4-dipyrrolidine-butyne) has been proposed[1,2] as a test drug against which anti-Parkinsonian agents may be screened and evaluated by their effect on the tremor induced by 'Tremorine' in mice. We have investigated the action of the compound in toads, mice, rats and guinea pigs; trials with larger animals, dogs and cats, were restricted by the shortage of 'Tremorine'.

The incidence and intensity of the symptoms caused by an effective, non-lethal dose of 'Tremorine' (*LD*5 or lower) varied with the species. Toads survived high doses (150 mgm./kgm.) without showing any constant characteristic symptoms other than transient excitation and occasional 'yawning', possibly indicating some discomfort in the respiratory passages. The mammals tested all showed a similar pattern of response. The *LD*50 for mice was of the order of 65 mgm./kgm. subcutaneous and 3–5 mgm./kgm. intraperitoneal; for rats 75 and 15 mgm./kgm. respectively, for guinea pigs 25 and 2·5 mgm./kgm. respectively. The effective dose, causing characteristic symptoms persisting for a few hours, was about one-quarter of the *LD*50. This dose was high enough to cause an occasional death. All animals tested exhibited within 15–30 min. of 'Tremorine' injection salivation, diarrhœa, occasional or persistent fine tremors of fore-limbs and head at the rate of 7–10 per sec., occasional single jerks or even convulsions, hypothermia and some analgesia. The present investigation was mainly concerned with the Parkinsonian-like tremors, and hypothermia was prevented by keeping the animals warm. The analgesic effect in mice has been dealt with by Chen[3] and electroencephalographic studies in cats have been made by Kaelber and Correll[4].

At the height of the drug effect, the animals were visibly ill, stretched out flat and unwilling to move, though responsive to stimuli and apparently fully aware of their surroundings. When moving, their limbs were often extended and stiff. Strutting of the fore-limbs or biting on the food tins or the wires of the cage may possibly be interpreted as behaviour aimed at negating the tendency to tremors.



a *b*

Fig. 1. From guinea pig killed 160 min. after injection of 70 mgm./kgm. 'Tremorine' subcutaneously. *a*, Intraventricular hæmorrhage; *b*, hæmorrhage on the surface of the cortex between gyri. Photomicrographs by courtesy of Dr. J. Mackiewicz

Typical persistent tremors were seen only in mice. In guinea pigs profuse salivation was most marked; in general, these animals appeared to be more prostrated than mice and tremors could often be demonstrated only when the animal was induced to move. The most impressive sign in rats was sanguineous lachrymation together with salivation and diarrhœa of the same nature as that caused by 0·5 mgm./kgm. carbamylcholine intraperitoneally.

The incidence of death at doses less than the *LD*50 was variable. Some animals died within 30 min. of injection, some died hours later after they appeared well on the way to recovery, while others died after 24–30 hr. in a prolonged coma, or in their sleep. Of those who died early, about 30 per cent had sudden, strong convulsions just before death, while the rest showed merely increasing sluggishness and weakness. Some animals recovered after they had already been recorded as dying. On post-mortem examination almost all animals, whether they died early or late, had injected brain vessels, and often visible hæmorrhages in the hemispheres or small blood clots in the ventricles (Fig. 1), 80 per cent had gross hæmorrhages as well, mainly subdural at the base of the brain or the brainstem, intrapericardial, intrapleural or intraperitoneal. A few retroperitoneal hæmorrhages were observed. It appeared that convulsions were present mainly in animals with cerebral hæmorrhages, but were absent with other internal hæmorrhages. Some intestinal hæmorrhages were also observed.

Repeated administration of sublethal doses of 'Tremorine' at intervals of 24–48 hr. did not produce convincing evidence of tolerance or cumulation, but it seemed that tremors and other motor disturbances were reduced while salivation and diarrhœa remained unaffected. (For this observation the animals were used only once, and kept at room temperatures above 16° C. throughout the experiment and the observation time.)

Atropine was found to be the most potent of all antagonists tried. In guinea pigs (*LD*50 of atropine sulphate about 400 mgm./kgm. intraperitoneally) 1–2 mgm./kgm. intraperitoneally prevented all symptoms if injected 20–30 min. before 'Tremorine', and abolished them within 10–15 min. if administered

after full development of the 'Tremorine' symptoms. The corresponding dose for the amino-alkyl ester 'Parpanit' was 25 mgm./kgm., and for the amino-alcohol 'Kemadrine' 17 mgm./kgm. The same effect was observed in mice. In rats no difference in the effect of antagonists could be noted whether the salivation and lachrymation were induced by 'Tremorine' or by carbamylcholine.

Everett's observation that the quaternary amino-alkyl ester 'Methantheline' (Banthine) in mice antagonizes the secretory symptoms preferentially without noticeably affecting the tremor was confirmed. The quaternary atropine-homologue propionylatropine-methylnitrate, 2–3 mgm./kgm. intraperitoneally, showed the same preferential antagonistic effect in mice, rats and guinea pigs. It must, however, be noted that in all animals recovering from 'Tremorine' alone, the tremors and other muscular symptoms persisted after diarrhœa and salivation had ceased, and could often be demonstrated on the following day when the animal had in all other respects completely recovered.

Another atropine homologue, tiglyl-pseudotropine ('Tigloidine'), which in clinical trials[5] had proved to be effective against Parkinsonian and related extrapyramidal disorders while lacking the *p*-sympathicolytic action of atropine, showed in weak doses only doubtful antagonistic effects against 'Tremorine'. At high doses of both compounds it even appeared that their toxic effects were potentiated; in some experiments all animals, mice, rats or guinea pigs which had received the combined medication convulsed and died, while the controls survived; the 'Tigloidine' controls without any noticeable symptoms, and the 'Tremorine' controls with the usual diarrhœa, salivation and muscular disturbances.

On these observations it does not appear to be justified to separate the tremor effect induced by low doses of 'Tremorine' in mice from the diarrhœa, salivation and other effects seen in all animals tried. These latter effects are notably absent in cases of Parkinsonism and related disorders. Furthermore, in animals practically recovered from 'Tremorine', tremors can usually be elicited if the animal is disturbed, while the typical Parkinsonian tremor is a tremor at rest. It would seem that 'Tremorine' is best classed with the group of *p*-sympathicomimetic drugs which all cause to a varying degree increased glandular secretion, gastro-intestinal motility and muscular over-activity ranging from tremors to convulsions. To all of these atropine and related compounds are common and highly effective antagonists. The fact that these antagonists are beneficial in Parkinsonism and related disorders may indicate that there is some relationship between these disorders, but it does not prove or even suggest that they are identical. It is doubted whether the 'Tremorine' and related tests can justifiably be presented as tests for anti-Parkinsonian drugs.

E. M. Trautner
S. Gershon
Departments of Physiology and Pharmacology,
University of Melbourne.
Feb. 23.

[1] Everett, G. M., Blockus, L. E., Shepherd, I. M., and Toman, J. E. P., *Fed. Proc.*, **15**, 420 (1956).
[2] Blockus, L. E., and Everett, G. M., *Fed. Proc.*, **16**, 283 (1957).
[3] Chen, G., *J. Pharm. Exp. Therap.*, **124**, 73 (1958).
[4] Kaelber, W. W., and Correll, R. E., *A.M.A. Arch. Neurol. Psych.*, **80**, 544 (1958).
[5] Trautner, E. M., and Noack, C. H., *Med. J. Austral.*, **1**, 751 (1957). Trautner, E. M., and Gershon, S., *Austral. Ann. Med.*, **70**, 286 (1958).

Excessive Uric Acid Production in the Human induced by 2-Ethylamino-1,3,4-thiadiazole

An excessive endogenous synthesis of uric acid is present in a substantial portion of patients with gout[1]. The precise nature of the metabolic defect responsible for such excessive production is not known, but may be the result of a defective homœostatic mechanism for control of purine synthesis. Evidence for the existence of such a mechanism in the normal human has come from the observation[2] that the simultaneous administration of 4-amino-5-imidazolecarboxamide suppresses *de novo* purine biosynthesis from glycine-^{15}N. Factors affecting uric acid synthesis in the human are therefore of special interest in regard to the study of gout.

The administration of 2-ethylamino-1,3,4-thiadiazole to human subjects as treatment for cancer results in a several-fold increase in both the excretion of uric acid in the urine and the serum urate-levels[3]. An increased purine biosynthesis was proposed to account for this observation, but an increased urate production and an elevation of serum urate has also been observed to accompany lysis of leukæmia cells during therapy[4], and destruction of other tissues could conceivably give similar results. A definitive experiment to rule out one of these alternative explanations was needed.

We have confirmed the original observation that the administration of ethylaminothiadiazole does result in a substantial increase in serum uric acid-levels accompanied by a two- to five-fold increase in urinary uric acid excretion. By measuring the incorporation of glycine-1-^{14}C into urinary uric acid in the presence and absence of ethylaminothiadiazole, we have been able to demonstrate that this increased uric acid excretion is accompanied by an increased incorporation of carbon-14 into uric acid (Fig. 1). Since uric acid arising primarily from degradation of tissue purines would tend to dilute the isotope in newly formed purines and give rise to a lower specific activity of uric acid, the present findings indicate a marked stimulation in the *de novo* synthesis of purines as a result of ethylaminothiadiazole action. The cumulative incorporation of the isotope into uric

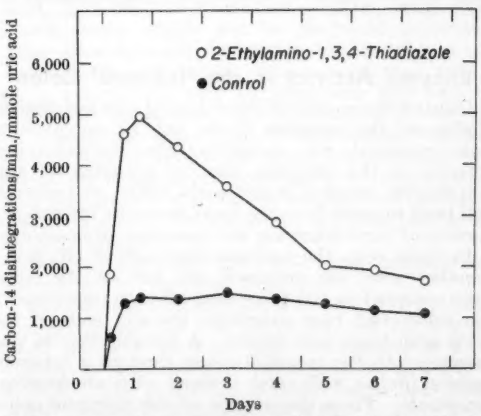


Fig. 1. Increased incorporation of glycine-1-^{14}C into urinary uric acid resulting from the administration of 2-ethylamino-1,3,4-thiadiazole to a non-gouty subject. 5 μc. of glycine-1-^{14}C was given orally at the beginning of each study and uric acid was isolated from the urine (ref. 2). A total of 350 mgm. of ethylaminothiadiazole hydrochloride was given in divided dose over a two-day period beginning one day before the second study

acid over a seven-day period was more than four times that observed in a similar period in the absence of ethylaminothiadiazole. The pattern and extent to which glycine-1-^{14}C was incorporated into uric acid in the presence of ethylaminothiadiazole with a maximum incorporation on the first day is very similar to the pattern of incorporation observed in many gouty subjects[5].

Some of the biological effects ascribed to ethylaminothiadiazole can be prevented by the simultaneous administration of nicotinamide. The carcinostatic action in experimental tumours in mice is diminished by nicotinamide[6]. We found that the increased serum urate-level and the increased uric acid excretion in the human could be completely prevented by administering a dose of nicotinamide four times the dose of ethylaminothiadiazole. This antagonism by nicotinamide was not apparent at equal doses of nicotinamide[3].

The increase in purine biosynthesis induced by ethylaminothiadiazole in the non-gouty human provides additional evidence favouring the view that purine biosynthesis is normally governed by a regulatory mechanism. A detailed explanation of the action of this drug should be of considerable value in defining the factors which normally operate to control the rate of purine biosynthesis in the human.

J. E. Seegmiller
Arthur I. Grayzel
Lois Liddle

National Institute of Arthritis
and Metabolic Diseases,
National Institutes of Health,
U.S. Public Health Service,
Bethesda, Maryland.
March 12.

[1] Benedict, J. D., Yü, T. F., Bien, E. J., Gutman, A. B., and Stetten, jun., DeW., *J. Clin. Invest.*, **32**, 775 (1953). Seegmiller, J. E., Laster, L., and Liddle, L. V., *Metabolism*, **7**, 376 (1958). Gutman, A. B., Yü, T. F., Black, H., Yalow, R. S., and Berson, S. A., *Amer. J. Med.*, **25**, 917 (1958).
[2] Seegmiller, J. E., Laster, L., and Stetten, jun., DeW., *J. Biol. Chem.*, **216**, 653 (1955).
[3] Krakoff, I. H., and Magill, G. B., *Proc. Soc. Exp. Biol. Med.*, **91**, 470 (1956).
[4] Gold, G. L., and Fritz, R. D., *Ann. Int. Med.*, **47**, 428 (1957).
[5] Wyngaarden, J. B., *J. Clin. Invest.*, **36**, 1508 (1957).
[6] Shapiro, D. M., Shils, M. E., Fugmann, R. A., and Friedland, I. M., *Cancer Res.*, **17**, 29 (1957).

Enzyme Activity in the 'Isolated' Colon

During the course of physiological and histological studies on the intestine of the cat, an opportunity was presented for comparing the histochemical activity of the enzymes alkaline phosphatase and non-specific esterase in the colon before and after it had been isolated from the small intestine for varying periods of time following the operation of ileostomy.

In eight cats, the terminal half-inch of the ileum together with the proximal one inch of the colon were removed in one piece, and after the contents of the colon had been expressed the cut ends of the colon and ileum were closed. A special cannula was inserted into the terminal ileum through a separate incision in its wall, and drained into an external receptacle. From the portion of the proximal colon removed at operation two small pieces were excised; one was fixed in cold 80 per cent alcohol for the histochemical demonstration of alkaline phosphatase by means of Kabat and Furth's[1] modification of Gomori's[2] 'cobalt sulphide' technique, and the other was fixed in cold absolute acetone for the histochemical demonstration of non-specific esterase by means of a modification[3] of Gomori's[4] 'Tween' technique, using 'Tween 40' as the substrate.

Table 1

No.	Before ileostomy		After ileostomy	Days
1	*A.P.*	+(+)	++	3
	Est.	+(+)	++	
2	*A.P.*	+	+(+)	5
	Est.	+(+)	+(+)	
3	*A.P.*	+	(+)	5
	Est.	(+)	(+)	
4	*A.P.*	+(+)	++	5
	Est.	+	++	
5	*A.P.*	(+)	++	5
	Est.	(+)	++	
6	*A.P.*	+(+)	++	5
	Est.	+(+)	++	
7	*A.P.*	+(+)	++	8
	Est.	++	++	
8	*A.P.*	+(+)	+	10
	Est.	(+)	(+)	

A.P., Alkaline phosphatase; *Est.*, non-specific esterase. The signs (+), +, +(+), ++ indicate the intensity of the enzyme activity.

After periods ranging from 3 to 10 days, the animals were killed, and pieces of tissue were removed from near the sutured end of the proximal colon for histochemical study of the activity of alkaline phosphatase and non-specific esterase, which was then compared with the activity shown in the biopsy specimens.

The histochemical reaction for alkaline phosphatase is particularly intense in the large intestine of the cat, and is of about equal intensity throughout its length[5]. The reaction is present in the intestinal contents, in the surface epithelium and in that of the crypts, extending into the latter for two-thirds or more of their length, then gradually fading in their deeper parts; this reaction occurs at the striated border of the cells of the surface and crypt epithelium and sometimes also in granules in the distal part of the cell cytoplasm. In addition, the reticular fibres around the crypt bases are positive. Non-specific esterase, as demonstrated by the 'Tween' technique, is localized in the cytoplasm of the epithelial cells lining the intestinal surface and approximately the upper one-third of the crypts, but the intestinal contents do not react[6].

Comparison of the enzyme activity in the specimens taken from the proximal colon before and after ileostomy was made by incubating all the sections in the same substrate bath for the same period of time. The reaction for alkaline phosphatase is so intense in the cat's colon that, to make this comparison, a very short incubation period had to be employed: after several trials, it was found that 2 min. was a suitable period to use. In the case of non-specific esterase, a long incubation period is needed with the 'Tween' technique to bring out a reaction; a period of 48 hr. was used, but, even after this time, differences in intensity of reaction were apparent.

The results of the investigation are summarized in Table 1. It is seen that, with the exception of two cases (Nos. 3 and 8), the reaction for alkaline phosphatase actually increased after ileostomy. Following the very short incubation period used, the histochemical reaction was somewhat incomplete, even in this strongly reacting tissue; it first appeared at sites of greatest activity, namely, in the middle and upper parts of many crypts, areas of the surface epithelium and sometimes in the contents within the crypts and intestinal lumen. Any increase in enzyme activity was shown by a heavier deposit of cobalt sulphide in

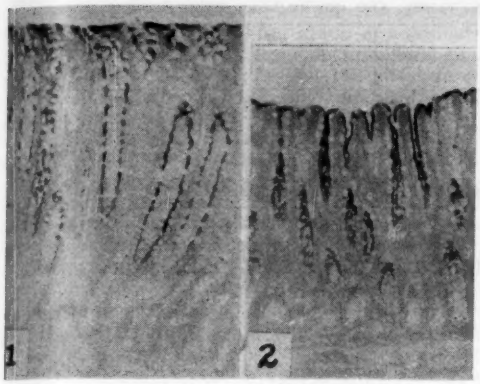


Fig. 1. Proximal colon, cat. Alkaline phosphatase reaction before ileostomy. 2 min. incubation. (× 75)

Fig. 2. Alkaline phosphatase reaction 5 days after ileostomy. The reaction has increased in extent and in intensity. Note shrinkage of the mucous membrane. 2 min. incubation. (× 75)

the reactive areas, and by an extension of the areas of reaction in the surface and crypt epithelium. An extension of the reaction into the deeper parts of the crypts, where the reaction is normally weaker than in the upper parts, was a particularly useful index of increased enzyme activity. Comparison of Fig. 1 with Fig. 2 (sections from No. 5 in Table 1) illustrates the difference in activity encountered before, and five days after, ileostomy.

Regarding non-specific esterase, it will be seen from Table 1 that in no case was there a decrease in enzyme activity after ileostomy; in four cases there was no obvious change, but in the other four (Nos. 1, 4, 5 and 6) there was actually an increase in activity.

It is concluded that, within the colon of the cat after 'isolation' by means of the operation of ileostomy, there is no diminution in the activity of the enzymes alkaline phosphatase and non-specific esterase, even after five or more days; on the contrary, the activity tends to increase.

It has been shown experimentally that alkaline phosphatase is secreted by the dog's colon[7]. The present histochemical results indicate that this is true for the cat, since the contents in the intestinal lumen and in the crypts are positive before and after ileostomy. However, the results show that the inflow of ileal contents is not a necessary factor either in maintaining the activity of the enzyme within the lining epithelium or in stimulating its continuous secretion. It is possible, however, that the quantity of alkaline phosphatase *secreted* is less in the 'isolated' colon, and that the increased activity in the epithelium indicates some degree of enzyme storage in the resting colon.

B. F. Martin

Department of Anatomy,
The University,
Sheffield, 10.
March 9.

[1] Kabat, E. A., and Furth, J., *Amer. J. Path.*, **17**, 303 (1941).
[2] Gomori, G., *Proc. Soc. Exp. Biol.* (N.Y.), **42**, 23 (1939).
[3] Martin, B. F., *J. Physiol.*, **119**, 24*P* (1953).
[4] Gomori, G., in "Methods in Medical Research", **4**, Sect. 1 (Yearbook Pub., Inc., Chicago, 1951).
[5] Martin, B. F., *J. Anat.* (Lond.), **85**, 140 (1951).
[6] Martin, B. F., *J. Anat.* (Lond.), **90**, 440 (1956).
[7] Kosman, A. J., Kaulbersz, J. W., and Freeman, S., *Amer. J. Physiol.*, **138**, 236 (1943).

Distribution of Haptoglobins in Native Venezuelans

Haptoglobins are globulins present in normal human plasma which are capable of combining and carrying hæmoglobin. First detected by Polonovsky and Jayle[1] in 1938, they became the object of detailed studies, mainly by French authors[2]. Smithies[3], in 1955, studying normal human serum by zone electrophoresis in a starch gel supporting medium, showed that it was possible to differentiate three groups easily when a certain amount of hæmoglobin was added to the serum. It was later demonstrated that this polymorphism was based on electrophoretic differences in the haptoglobins[4].

In 1958, Allison, Blumberg and Rees[5] studied the distribution of haptoglobins in English subjects, Spanish Basques and Negroes from Nigeria. By comparing these results with previous studies[6,7] made on Canadian and United States subjects and negroes from Liberia and the Ivory Coast[7], they concluded that the distribution of haptoglobins might well have anthropological significance. Since the Venezuelan population is derived mainly from various Indian racial stocks, different European groups (especially Basques and other Spanish branches) and African Negroid populations, a study of such a population should help prove such an assumption. The present communication gives the first results obtained by studying the distribution of different types of haptoglobins in the native population of Venezuela.

A study was conducted on 208 adult persons, aged 18–50, apparently normal, most of whom were voluntary donors of the Hospital Universitario de Caracas Blood Bank. The majority was from Caracas and the rest came from nearby districts and such distant places as San Cristóbal (Estado Táchira), San Fernando (Estado Apure) and Güiria (Estado Sucre). Since donors are generally male, only 27 female subjects were studied.

Blood was obtained by venipuncture and left clotting for 2–3 hr. Sera not immediately used were kept frozen until the time of use, when enough hæmoglobin solution was added to saturate haptoglobins.

In order to distinguish the various groups of haptoglobins, zone electrophoresis in a starch gel was used, according to Smithies's method[6], with slight modifications which will be described separately. After electrophoretic migration the starch gel slab was divided horizontally into two halves. One of them was stained with amidoschwarz 10*B* and the other with benzidine[8]. By this method it was easy to classify the right type of haptoglobin for each sample, and in order to check this classification those pertaining to one group were again studied together. This also gave a comparative evidence of previously described minor differences in subjects having the same phenotype[6,8,9].

Both the ABO and Rh blood group systems were determined by standard techniques.

The material studied gave a frequency of 27·40 per cent for phenotype 1–1, 17·79 per cent for 2–2 and 54·81 per cent for 2–1. This corresponds to a frequency for gen Hp^1 of 0·54805 and for gen Hp^2 of 0·45195. The χ^2 test for 'goodness of fit' was 0·011. With 1 degree of freedom, P is 0·91.

No relation was found, in analysing our results (Table 1), between haptoglobins and blood groups. This agrees with other authors[10,11].

Table 2 shows the distribution of different types of haptoglobin in the populations hitherto studied, to

Table 1. Distribution of Haptoglobins according to Sex, ABO and Rh Blood Group Systems

Haptoglobin phenotypes	No.	Sex (per cent)		ABO (per cent)				Rh (per cent)	
		Male	Female	O	A	B	AB	+	−
Hp 1–1	57	87·72	12·28	42·10	38·60	12·28	7·02	96·49	3·51
Hp 2–1	114	89·47	10·53	54·39	29·82	11·4	4·39	90·35	9·65
Hp 2–2	37	78·38	21·62	48·65	45·95	2·70	2·70	86·49	13·51

Table 2. Distribution of Haptoglobin Types in Various Populations

Population	Reference	No. tested	0–0		1–1		2–1		2–2	
			No.	(per cent)	No.	(per cent)	No.	(per cent)	No.	(per cent)
Caucasoid :										
Canadian (Toronto)	6	49	0	0	10	21·1	25	50·5	14	28·4
American (Chicago)	7	54	0	0	16	11·1	29	53·7	19	35·2
Swedish (Lund)	11	46	0	0	7	15·0	23	50·0	16	35·0
British (Oxford)	5	218	6	2·7	22	10·1	121	55·5	69	31·7
Basque	5	107	1	0·9	15	14·0	49	45·7	42	39·3
Negroid :										
Africans (Liberian and Ivory Coast)	7	142	0	0	69	48·7	60	42·3	13	9·1
African (Nigeria)	5	99	32	32·3	53	53·5	11	11·1	3	3·0
Hybrid :										
Venezuelan (Caracas)	—	208	0	0	57	27·40	114	54·80	37	17·80

which our own results have been added. When an anthropological separation is made, the material investigated by us appears to be the first hybrid population studied.

It appears from this Table that our results are fundamentally different, in many respects, from those obtained by other authors. The frequency obtained for phenotype 1–1 (27·40 per cent) is much higher than that obtained for Caucasoid populations but lower than that of Negroids. This incidence might indicate in what percentage the African race enters into our native population. On the other hand, the frequency of phenotype 2–2 found in the Venezuelan population (17·79 per cent) is much higher than that found in African populations but lower than that of Caucasoids, which might indicate the participation of the European element in our hybrid population. Our results seemingly confirm the anthropological importance of haptoglobin frequency. Since the Indian population has not been studied before it has not been included. It is now being investigated and will be the subject of a further report.

It is of importance that the presence of phenotype 0–0 was not noticed in our material. This phenotype was not originally described by Smithies[6], but its existence was postulated by Allison, Blumberg and ap Rees[5] in their studies of samples from Nigeria, and also by Harris, Robson and Siniscalco[12] in their studies of 107 families. Keeping in mind the high percentage in which the African factor enters into the constitution of the present native population of Venezuela, this negative result is of primary importance since it indicates that the African populations taken to Venezuela in the days of the slave trade may have been biologically different from the present population of Nigeria. If future investigations should confirm the absence of this phenotype in the normal population of Venezuela or any other South American country, these results would confirm those of Sutton *et al.*, who apparently found no individual differences from the three phenotypes described by Smithies[3,6]. This is contrary to the idea of Allison *et al.* that the series of Sutton *et al.* possibly included subjects of group 0–0 in group 1–1. If this explanation is rejected, one would have to conclude that both the samples from Nigeria examined by Allison *et al.* and the material studied by Harris *et al.* comprised persons who for some subclinical process (hæmolytic anæmia, pernicious anæmia, hepatitis, etc.) showed a temporary absence of haptoglobins (acquired ahaptoglobinæmia) instead of a permanent absence (congenital ahaptoglobinæmia). Careful analysis of the results of Allison *et al.* (Table 2) seems to show that the great proportion of phenotype 0–0 found reduces the frequency of phenotype 2–1. This phenotype, with the most haptoglobins, is more easily made to disappear by any of the pathological processes mentioned above.

We are indebted to Mr. Alfredo Pansini for helping in the translation of the Spanish text.

M. L. Gallango de Rodríguez
Tulio Arends

Instituto Venezolano de Investigaciones Científicas,
Sección Medicina,
Caracas-Venezuela.

March 3.

[1] Polonovski, M., and Jayle, M. F., *Bull. Soc. Chim. Biol.*, **21**, 66 (1939).
[2] Jayle, M. F., and Boussier, G., *Expos. Ann. Biochim. Med.*, **17**, 157 (1955).
[3] Smithies, O., *Nature*, **175**, 307 (1955).
[4] Smithies, O., and Walker, N. F., *Nature*, **178**, 694 (1956).
[5] Allison, A. C., Blumberg, B. S., and ap Rees, *Nature*, **181**, 824 (1958).
[6] Smithies, O., *Biochem. J.*, **61**, 629 (1955).
[7] Sutton, H. E., Neel, J. V., Binson, G., and Zuelzer, W. W., *Nature*, **178**, 1287 (1956).
[8] Allison, A. C., and ap Rees, W., *Brit. Med. J.*, ii, 1137 (1957).
[9] Bearn, A. G., and Franklin, E. C., *Science*, **128**, 596 (1958).
[10] Smithies, O., and Walker, N. F., *Nature*, **176**, 1265 (1955).
[11] Laurell, A. B., and Grubb, R., *Vox Sanguinis*, **2**, 312 (1957).
[12] Harris, H., Robson, E. B., and Siniscalco, M., *Nature*, **182**, 1324 (1958).

Pathways and the Physiological Site of Anserine Formation

It is clear from *in vivo*[1] and *in vitro*[2] studies that vertebrate skeletal muscle contains an enzyme that can condense 1-methylhistidine and β-alanine (in the presence of magnesium and adenosine triphosphate) to yield anserine. However, two other paths of anserine biosynthesis have been suggested[1]: β-alanyl transfer from carnosine to methylhistidine, and the direct methylation of carnosine. The former possibility has not yet been investigated. The latter alternative is the subject of the present communica-

tion. While methyl-labelled methionine was shown to supply effectively the methyl group of anserine[3,4] *in vivo* it was not evident from such experiments whether N-methylation occurred at the histidine or carnosine stage, nor was the physiological site of anserine formation revealed.

The investigations of Cantoni[5] and of Stekol, Anderson and Weiss[6] have disclosed that S-adenosylmethionine is the direct methyl group donor in the synthesis of creatine (from guanidoacetic acid) and choline (from dimethylaminoethanol) in the liver. It was of interest, accordingly, to determine whether the activated methionine derivative could likewise transfer its methyl radical to histidine or carnosine in the presence of tissue preparations. For this purpose we prepared S-adenosyl-L-methionine, labelled in its methyl with carbon-14, by a biosynthetic method[6]. An extract of chick pectoral muscle[2], found to be active in the biosynthesis of carnosine and anserine, was tested under conditions similar to those employed for the synthesis of choline and creatine by liver homogenates[6], except that our substrate concentrations were somewhat higher. Following a 2-hr. incubation at 37° C. (Fig. 1), the protein was precipitated by addition of 1·5 ml. of ethanol to the reaction mixture, and heating to boiling. After centrifugation, aliquots of the supernatant solution were subjected to paper chromatographic analysis (Whatman No. 1). The solvent was *t*-butanol–formic acid–water (70 : 15 : 15). After 24 hr. the methylhistidine and anserine regions were about 3–5 cm. from the origin, while the excess isotopic methionine and its adenosyl derivative (actually decomposed, apparently to S-methyladenosine) migrated much farther along the paper. The desired radioactive areas were cut out and measured in a windowless gas-flow Geiger counter. All values were corrected for the small background radioactivities found in parallel experiments with inactivated enzyme preparations. Fig. 1 shows that even in the absence of non-labelled substrate about 1 per cent of the carbon-14 of adenosylmethionine was used for transmethylation. This result can very likely be attributed to the considerable concentration of carnosine in the muscle extract employed. With the addition of excess histidine, the utilization of adenosylmethionine reached 2 per cent; while a value of 3 per cent was attained when the concentration of added carnosine was 9 μmoles/ml. It can be seen that methionine itself was ineffective for methylation. Apparently this amino-acid was not activated under the conditions employed.

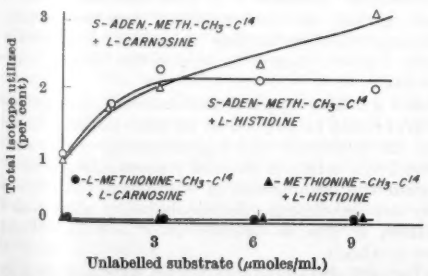


Fig. 1. Comparison of methione and its adenosyl derivative as methyl donors for histidine and carnosine. The incubation mixture (0·5 ml. final volume, pH 7·4) contained 0·3 ml. of centrifuged 1 : 4 muscle homogenate, histidine or carnosine in varying concentrations, and either L-methionine-CH_3-^{14}C or S-adenosyl-L-methionine-CH_3-^{14}C (each with an activity of 0·1 mc./m.mole) at a level of 3·5 μmoles/ml.

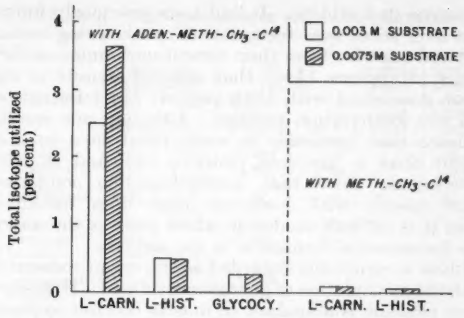


Fig. 2. Relative rates of methylation of histidine and carnosine by ethanol-precipitated protein fraction of muscle. The incubation mixture (0·5 ml.) contained 8 mgm. protein and 3·5 μmoles labelled methylating agents per ml., in addition to histidine or carnosine. The conditions of incubation and of subsequent analysis were those of Fig. 1 except that *n*-butanol–ethanol–water (4 : 1 : 1) was used for the chromatographic isolation of creatine (found 2–4 cm. from the origin after 24 hr.)

Similar results were obtained (Fig. 2) when a protein fraction of chick pectoral muscle extract, precipitated between 20–60 per cent ethanol concentration[7], was used as source of enzyme. The rate of methylation of carnosine relative to that of histidine is accentuated in Fig. 2, as compared with the corresponding ratio with the crude muscle extract. Here again, when free isotopic methionine was used, no significant radioactivity was found in the methylhistidine or anserine regions of the chromatograms. Our highest values obtained with muscle are comparable in magnitude to those for creatine and choline synthesis with liver homogenates (about 4–5 per cent utilization of adenosylmethionine in 2 hr.)[6].

The results with muscle enzyme were confirmed by the use of isotopic histidine and carnosine, together with unlabelled S-adenosylethionine. The availability of these compounds and the ease of chromatographic separation of ethylated products (as compared with methylated) from the parent histidine and carnosine suggested the experiment of Table 1. Following incubation, chromatography was performed with 2 : 1 pyridine/water for 24 hr. Histidine and carnosine migrated 19–21 cm., while ethylhistidine and ethylcarnosine were located at 24–26 and 25–27 cm., respectively, from the origin. (Synthetic 1-ethylhistidine[7] was used as a reference standard.) Because of the very soft β-rays of tritium it was necessary to elute the ethylcarnosine region from the paper, and to evaporate the eluate on a planchet, in order to measure accurately the radioactivity (in the gas-flow counter). Table 1 shows that, as in the methylation experiments, carnosine was N-alkylated more readily than histidine.

In experiments performed with chick liver homogenates we could not detect significant utilization of labelled adenosylmethionine for transmethylation of

Table 1. ADENOSYLETHIONINE AS AN ALKYLATING AGENT FOR HISTIDINE AND CARNOSINE

The conditions were those of Fig. 2, except that the incubation mixture contained 2 μmoles/ml. of non-isotopic S-adenosyl-L-ethionine and 3·5 μmoles/ml. of either DL-histidine-2-^{14}C (0·5 mc./m.mole) or L-carnosine-^{3}H (about 1 mc./m.mole). The latter compound was prepared by tritium-recoil labelling (ref. 8), and purified by paper chromatography with 2 : 1 pyridine–water

Isotopic substrate	Percentage of total radioactivity utilized for transethylation
L-Histidine-2-^{14}C	0·75
L-Carnosine-^{3}H	2·15

carnosine or histidine. It had been previously found that liver slices were incapable of synthesizing carnosine and anserine from their constituent amino-acids[9]. Hence it appears likely that skeletal muscle is the tissue concerned with both peptide bond formation and the methylation process. Although our results indicate that carnosine is methylated to a greater extent than is histidine, both *in vivo* and *in vitro* experiments show that methylhistidine condenses more readily with β-alanine than does histidine. Thus it is difficult to decide which path is the major one for anserine formation in the animal.

Liver is commonly regarded as the organ concerned with the methylation of guanidoacetic acid. However, since creatine is abundant in muscle but not in liver, it seemed of interest to determine whether muscle tissue could also form creatine from its precursor. Fig. 2 shows that this process was negligible in circumstances which promoted relatively rapid methylation of carnosine by adenosylmethionine. If it is assumed that the conditions employed for the detection of creatine synthesis were optimal, the results suggest that the transmethylating system of muscle is rather restricted in its specificity, and may function primarily in the formation of anserine.

This work was supported by a grant-in-aid of the Muscular Dystrophy Associations of America.

We wish to thank Dr. J. A. Stekol of the Institute for Cancer Research, Philadelphia, and Dr. G. L. Cantoni, National Institute of Mental Health, Bethesda, for kindly supplying samples of adenosylmethionine and -ethionine, together with helpful information.

Theodore Winnick
R. E. Winnick

Section of Biochemistry,
Weizmann Institute of Science,
Rehovoth, Israel.
Feb. 26.

[1] Harms, W. S., and Winnick, T., *Biochim. Biophys. Acta*, **15**, 480 (1954).
[2] Winnick, T., and Winnick, R. E., *Biochim. Biophys. Acta*, **31**, 47 (1959).
[3] Schenck, J. R., Simmonds, S., Cohn, M., Stevens, M. C., and du Vigneaud, V., *J. Biol. Chem.*, **149**, 355 (1943).
[4] McManus, I. R., *J. Biol. Chem.*, **225**, 325 (1957).
[5] Cantoni, G. L., *J. Biol. Chem.*, **189**, 203, 745 (1951).
[6] Stekol, J. A., Anderson, E. I., and Weiss, S., *J. Biol. Chem.*, **233**, 425 (1958).
[7] Winnick, T., and Winnick, R. E., *Bull. Soc. Chim. Biol.*, **40**, 1727 (1958).
[8] Rowland, F. S., and Wolfgang, R., *Nucleonics*, **14**, 58 (1956).
[9] Winnick, T., and Winnick, R. E., *Biochim. Biophys. Acta*, **23**, 649 (1957).

A New Hæmagglutinating Substance in the Gm System, Anti-Gm

A system of inherited serum groups has been described by Grubb and Laurell[1]. Sera from certain patients with rheumatoid arthritis agglutinated Rh-positive red cells which had been coated with selected incomplete anti-D. The agglutination was inhibited by sera from about 60 per cent of normal individuals, who were said to be Gm(a+) because the inhibiting substance was located in the gamma-globulin fraction of serum. This substance was inheritable, determined by the gene Gm^a, which expressed itself in single or double dose. The corresponding allele was called *Gm*.

Among 320 unrelated Norwegians, the following frequencies were found[2]:

Phenotypes		Genes	
Gm(a+)	194 (60·63 per cent)	Gene Gm^a	0·3726
Gm(a−)	126 (39·37 per cent)	Gene *Gm*	0·6274

We investigated sera from 65 patients with rheumatoid arthritis presenting a positive Waaler-Rose's test, and found one serum (J.K.) which contained a substance antithetic to anti-Gm^a.

Group O red cells of genotype C+ D+ E+ c+ were coated with a selected sample of incomplete anti-D. The anti-D serum was of type Gm(a−). After washing, the cells were agglutinated by serum J.K. diluted up to 1 : 256, giving a score[3] of 29. The reactions were run on a slide as previously described[2], and the degree of agglutination was read microscopically. The agglutination was inhibited by pooled human gamma-globulin and by 97 (81·5 per cent) of 119 sera from unrelated individuals. For typing, the rheumatoid serum was set up in serial twofold dilutions. To one drop of each dilution one drop of the serum to be tested (diluted 1 : 8) and one drop of a 0·3 per cent suspension of coated red cells were added. Most of the sera (81·5 per cent) inhibited the agglutinating substance of serum J.K., as shown by reduction of agglutination scores to between 0 and 8. In contrast, normal sera giving scores of 21–29 were considered non-inhibiting. Sera obtained from venous blood samples always gave clear-cut reactions, whereas intermediate reactions (score 10–15) were met with in 4 of the samples of capillary blood from children in a family material.

The specificity of the agglutinating substance in serum J.K. was ascertained by the following observations:

1. The investigation of sera from 119 unrelated individuals (including the parents in the family material) gave the following result:

	Inhibition of agglutination	No inhibition of agglutination
Gm(a+) 73	51	22
Gm(a−) 46	46	0

The probability that the hæmagglutinating substance in serum J.K. is unrelated to the Gm system is $p < 0{\cdot}0001$ ($\chi^2 = 16{\cdot}99$ for 1 degree of freedom).

Among the 73 Gm(a+) individuals, 56 were probably heterozygous (Gm^a/Gm) and 17 homozygous (Gm^a/Gm^a), as calculated from the gene frequencies. Actually, 51 of the sera inhibited the agglutinating substance in serum J.K., while 22 sera did not. The difference between the observed and expected values is not significant ($\chi^2 = 1{\cdot}92$ for 1 degree of freedom).

2. Sera from the members of 25 families with 66 children were studied.

Among the children, 26 were of type Gm(a−). Sera from 25 of them inhibited the agglutinating substance in serum J.K., while one serum showed an intermediate reaction. If one assumes that the serum giving an intermediate reaction really is inhibiting, the probability that this distribution is merely due to chance is $P = 0{\cdot}025$ (Fisher's exact method).

Based on Gm^a type determinations, the genotype Gm^a/Gm could be proved in 24 individuals. Of these sera, 22 inhibited the agglutinating substance in serum J.K., while 2 showed intermediate reactions. If again the assumption is made that the two sera really are inhibiting, the probability that this distribution is due to chance is $P = 0{\cdot}043$ (Fisher's exact method).

When all children (4 or more) in matings of type Gm(a+) × Gm(a−) were Gm(a+), the Gm(a+) parent was expected to be of genotype Gm^a/Gm^a. Similarly, when 4 or more Gm(a+) children were found in a mating of type Gm(a+) × Gm(a+), one of them was expected to be of genotype Gm^a/Gm^a.

On this basis, 6 individuals in the family material were expected to be of genotype Gm^a/Gm^a. Sera from 5 of them did not inhibit the agglutinating substance in serum J.K., while one serum did so. The probability that this is merely due to chance is $P = 0{\cdot}05$ (Fisher's exact method).

In seven families, one or more children were thought to be of genotype Gm^a/Gm^a because their sera did not inhibit the agglutinating substance in serum J.K. In all these matings, both parents were of type Gm(a+). The probability that this is due only to chance is $P = 0{\cdot}00091$.

The fourth intermediate reaction was observed in a child of type Gm(a+), whose genotype could not be predicted.

From these observations, it is concluded that the agglutinating substance in serum J.K. reveals the substance determined by the gene *Gm*. The serum is therefore called anti-Gm in accordance with previous designations[2] of sera related to the Gm system. The term anti-Gm does not imply that the agglutinating substance is necessarily the result of previous immunization; it is simply a designation based on the observation that the agglutinating substance in serum J.K. reacts with the Gm substance.

MORTEN HARBOE

Institute for Forensic Medicine,
Institute for Thrombosis Research,
Institute for Human Genetics,
University of Oslo.

Mar. 3.

[1] Grubb, R., and Laurell, A.-B., *Acta Path. Microbiol. Scand.*, **39**, 390 (1956).
[2] Harboe, M., and Lundevall, J., *Acta Path. Microbiol. Scand.*, **45**, 357 (1959).
[3] Stratton, F., and Renton, P. H., "Practical Blood Grouping" (Oxford, 1958).

Exploratory Physicochemical Studies on the Sense of Taste

IN the past, many chemists have tried to account for the tastes of substances in terms of their chemical structures. Although limited correlations have been found, no rigid, widely applicable generalization has evolved. In view of the poor success in this direction, it is apparent that the relationship is very complex. With the belief that taste, as well as other physiological responses, will be understood eventually in terms of the molecular properties of substances, a deliberate search was begun in this laboratory to identify these properties for the sense of taste.

Our general plan of attack has been twofold[1]. One approach is to postulate a mechanism by which the sapid substance can trigger a nerve impulse to the brain to be recognized as a taste sensation and then to examine the molecular properties related to this mechanism. The second approach is to search for correlations between taste and molecular properties with the hope that one or more will be found to give a clue to the mechanism of taste stimulation. Space does not permit an elaboration of the reasoning followed in choosing the properties to be measured. Furthermore, since no positive generalizations have been discovered, it is sufficient to report only the experimental results.

A specific group of compounds was selected the tastes of which range from tasteless to 4,000 times as sweet as sucrose. The relative sweetnesses were determined by a taste panel under the supervision of Drs. Frank Pilgrim and Howard Schutz of the Quartermaster Food and Container Institute, U.S. Army, Chicago. Several isomeric pairs were chosen, including optical isomers, in order to have a critical test for any relationships discovered. Attention was confined to the sweet taste in order to keep the number of variables to a minimum and because structural modifications can be made to elicit a wide variation in intensity of response.

Table 1. COMPOUNDS STUDIED AND THEIR PROPERTIES

Compound	Relative sweetness		$E_{1/2}$ [a] (volts)		Wet-melting point depression[b] (° C.)	Surface[c] tension at $10^{-4}M$ (dynes/cm.)
	This study	Lit.[d]	This study	Lit.[d]		
Sucrose	1·00	1·00	−0·85*			62·0
Fructose	1·15	1·73	−1·86	−1·80		60·8
		1·05				
α-D-Glucose	0·64	0·74	−1·56	−1·58	78	55·4
		0·35				
Galactose	0·59	0·32	−1·55	−1·57		
Mannose	0·59		−1·53			
Lactose	0·33	0·16				
Sucrose octaacetate	bitter		−1·05*			
Glucose penta-acetate	bitter		−1·02*			
α-Methyl glucoside	0·52		none			
Stevioside	280	*c.* 300				53·7
Saccharine	306	425–675	−1·73†	−1·94 (*p*H 10)		
		450–500		−1·25 (*p*H 2)		
Dulcin	91					
Sucaryl	33·8	*c.* 30				
2-CH_3O-5-NO_2-aniline	167	220–330	−0·43†		10	57·5
		2,150				
4-CH_3O-3-NO_2-aniline	tasteless		−0·42†		9	54·0
2-C_2H_5O-5-NO_2-aniline	703	950–1,400	−0·48†			
		830				
4-C_2H_5O-3-NO_2-aniline	tasteless					
2-*n*-C_3H_7O-5-NO_2-aniline		4,100				
		3,100				
2-HO-5-NO_2-aniline	20·5	120–200	−0·48†		60	
4-HO-3-NO_2-aniline	not sweet		−0·31		20	
2-CH_3-5-NO_2-aniline	298	sweet	−0·40†			
4-CH_3-3-NO_2-aniline	not sweet		−0·42†			
2-CO_2H-5-NO_2-aniline	not sweet		−0·34			
2-Cl-5-NO_2-aniline	375	400	−0·35			7
		700				
4-Cl-3-NO_2-aniline	not sweet		−0·40			9
2-Br-5-NO_2-aniline	714	800	−0·33		10	63·7
		1,750				
4-Br-3-NO_2-aniline	not sweet		−0·34		11	66·9
m-Nitro-aniline		40	−0·38	−0·43		
Nitro-benzene			−0·42	−0·48 (*p*H 5·7)		
D-Trypto-phan	222	sweet				
DL-Trypto-phan	111	sweet				
L-Trypto-phan	not sweet	not sweet				
D-Aspar-agine	2·1	sweet				
L-Aspar-agine	not sweet	not sweet				

[a] $E_{1/2}$, the half-wave reduction potential with reference to the saturated calomel electrode, measured with a Sargent–Heyrovsky model XII recording polarograph at 37·1 ± 0·03° C. and *p*H 6·6.

[b] Wet-melting point depression, namely, the difference between the melting point of a substance when dry and when in contact with water, and is used as a measure of the degree of association of the substance with water.

[c] Measurements made by Mr. Andrew Wims.

[d] Literature sources of data cited in Table 1 may be obtained from the authors.

* Conceivably the value for an impurity; however, every precaution was taken to use materials of highest purity. The same value was obtained, for example, using sucrose of grade National Bureau of Standards Calorimetry Standard, m.p. 171–174° C., and Fisher Scientific Certified Reagent, m.p. 182–184° C.

† Gelatine added as a suppressor.

Table 2. Adsorption Characteristics of Sweet and Tasteless Isomeric Nitroanilines

Compound	Wave-length for analysis	Molar absorbancy index
	(mμ)	
(I) 2-Bromo-5-nitroaniline (sweet)	247	9,840
(II) 4-Bromo-3-nitroaniline (tasteless)	237	16,320
(III) 2-Methoxy-5-nitroaniline (sweet)	253	12,320
(IV) 4-Methoxy-3-nitroaniline (tasteless)	228	16,900

Compound	Adsorption on charcoal			Adsorption on hair		
	a	$1/n$	W	a	$1/n$	W
I	0·340	0·830	0·0743	0·0436	0·417	0·0095
II	0·248	0·671	0·0538	0·0495	0·484	0·0108
III	0·269	0·732	0·0585	0·0244	0·494	0·0041
IV	0·186	0·745	0·0403	0·0125	0·275	0·0021

In the search for correlations between taste and molecular properties, a few properties which could affect the taste of a compound were studied first. Although some of these properties had been measured by previous investigators, the present work differs in that all measurements were made by the same group of investigators on compounds the tastes of which had been evaluated by the same panel. The results are given in Table 1.

Our second approach for finding a clue to the molecular properties responsible for characteristic tastes is to consider mechanisms by which sapid substances might trigger nerve impulses to the brain. Table 2 contains the results of a search for a correlation between the sweet taste and adsorption on a certain surface. Charcoal was used first in order to work out the experimental procedure. The hair was that of a child, washed in a nonionic detergent, and air-dried at room temperature. Two types of tongue tissue were used in unreported experiments but the results were not reproducible. The results in Table 2 fit the Freundlich equation :

$$x = \alpha c^{1/n}$$

where x represents millimoles of adsorbate held per unit weight of adsorbent ; c the concentration of adsorbate remaining unadsorbed at equilibrium in moles/litre ; α and n are constants ; while, in Table 2, W is the weight of substance adsorbed at the concentration of 0·01 gm./100 gm. solution.

Experiments were done to detect any special interaction between sweet compounds and certain biological substances. The results from one type of experiment are given in Table 3, from which no significant difference is observed between a sweet compound and its tasteless isomer. The numbers I and II refer to the compounds in Table 2. Other experiments were conducted to learn whether or not certain components common to enzyme systems are involved in taste chemireception. Thus, sweet solutions of 2-methoxy-5-nitroaniline and salty sodium chloride solutions were tasted in a helium atmosphere. Other than a sort of warming sensation, no significant difference in taste from that in air was observed. Sweet solutions of 2-methoxy-5-nitroaniline were tasted when diluted with solutions of magnesium chloride, manganous chloride, or water. The solutions containing magnesium or manganous ions were bitter and sweet, and magnesium ion could not be distinguished from manganous ion by taste. Then, to see whether or not the sapid substance interacts directly with an enzyme, solutions of a sweet compound were tasted in the presence of certain enzyme inhibitors. For example, several aliquots of a sweet solution of 2-methoxy-5-nitroaniline were diluted separately with water, or with solutions of sodium azide, potassium fluoride, sodium iodoacetate, or sodium cyanide. These solutions were mixed and tasted in random order several times without their identity being known to the taster (L. N. F.). Azide enhanced the sweetness of the nitroaniline, but fluoride and iodoacetate diminished the sweetness a very small amount, iodoacetate gave an iodine flavour, and cyanide was very bitter, outweighing the sweet taste. However, none of these recognized enzyme inhibitors obliterated the sweet taste irreversibly. This fact suggests that the taste receptor surface is not that of an enzyme.

Thus several physical properties which conceivably could be factors determining the tastes of substances have been measured. Also, various possible mechanisms for stimulus-receptor interactions have been examined. Although no positive generalizations have been found, and these experiments have been exploratory, the results serve as guides for future work in pursuing the identity of the molecular proper-

Table 3. Light Absorbancies of Various Solutions Alone and When Mixed

Compound	A^a	Sum	Change when mixed (per cent)	Compound	A^b	Sum	Change when mixed (per cent)
I	0·438			II	0·182		
Saliva	0·444	0·882		Saliva	0·200	0·382	
Mixture	0·845		−4·2	Mixture	0·360		−5·75
I	0·438			II	0·428		
Bovine gamma globulin	0·064	0·502		Bovine gamma globulin	0·135	0·563	
Mixture	0·486		−3·2	Mixture	0·534		−5·5
I	0·428			II	0·418		
ATP	0·145	0·573		ATP	0·078	0·496	
Mixture	0·563		−1·8	Mixture	0·478		−3·6
I	0·408			II	0·398		
ATP	0·112			ATP	0·048	0·446	
Mg^{2+}	0·000	0·520		II, ATP, Mg^{2+}	0·452		
Mixture	0·528		*c.* 0	II, ATP	0·452		*c.* 0
I	0·433			II	0·423		
Globin*	0·051	0·484		Globin	0·053	0·476	
Mixture	0·449		−7·8	Mixture	0·452		−5·3
Mixture + Mg^{2+}	0·452			Mixture + Mg^{2+}	0·454		

[a] Measurements made at the $\lambda_{max.}$ of I.
[b] Measurements made at the $\lambda_{max.}$ of II.
* A filtered colloidal solution of globin.
ATP, adenosine triphosphate.

ties responsible for the characteristic tastes of substances. It is to be realized that the nature of the stimulus-receptor interaction is unknown and these exploratory-type experiments constitute an attempt to find a clue on a gross level.

This work was undertaken in co-operation with the Quartermaster Food and Container Institute for the Armed Forces, Quartermaster Research and Engineering Command, U.S. Army.

AETIUS R. LAWRENCE
LLOYD N. FERGUSON

Chemistry Department,
Howard University,
Washington, D.C.

[1] *J. Chem. Educ.*, **35**, 436 (1958).

Energy Compensation in the Crabtree Effect with Ehrlich Ascites Carcinoma Cells

RECENTLY, Quastel and Bickis[1] published a very interesting and stimulating article concerning the energy relationships in several intact cell types, including the Ehrlich ascites carcinoma cell. They determined the ratio of lactate produced to the difference between oxygen consumed in the cell system with and without added glucose (average value 5·8). The assumption was made that the amount of adenosine triphosphate produced in the two systems was the same. They reasoned that if the total production of energy remained constant in the two cell systems, the ratio phosphorus/oxygen would be about 3.

Table 1. ENERGY RELATIONSHIP BETWEEN GLYCOLYSIS AND THE DEPRESSION OF RESPIRATION OF THE CRABTREE EFFECT

Compound (μM per ml. cells)	With glucose	Endogenous	Lactate / Δ Oxygen
Oxygen utilized	16·3	27·7	
Lactate accumulated	57·2	—	5·0
Adenosine triphosphate present	1·60	1·65	(P : O = 2·5)

Incubation system: 12·4 volume per cent of tumour cells in phosphate Locke's solution, *p*H 7·4; 30° C. for 15 min.; glucose 51·7 μM per ml. cells.

We recently published observations[2] on the Ehrlich ascites cells which show that the adenosine triphosphate content of the endogenous- and the glucose-containing cell is the same, except for an initial brief period of equilibration. This means that glycolysis does produce an amount of energy equivalent to the energy lost by the decreased oxygen consumption, provided the utilization of adenosine triphosphate remains constant. Furthermore, Table 1 shows that the accumulation of lactate could nearly account for the decrease in oxygen consumption in terms of production of adenosine triphosphate, assuming a phosphorus/oxygen ratio of approximately 3 (2·5) and glycolytic production of 2 molecules of adenosine triphosphate per glucose molecules. These results are in good agreement with Quastel and Bickis's hypothesis.

K. H. IBSEN
E. L. COE
R. W. MCKEE

Department of Physiological Chemistry,
University of California,
Los Angeles 24.

[2] Quastel, J. H., and Bickis, I. J., *Nature*, **183**, 281 (1959).
[3] Ibsen, K. H., Coe, E. L., and McKee, R. W., *Biochim. Biophys. Acta*, **30**, 284 (1958).

PATHOLOGY

Homologous Species Neoplastic Antibodies

Prehn and Main[1], in a review of the subject, state that the demonstration of circulating cytotoxins is much simpler when heterologous tissue transplants are used as antigens than when homologous tissues are used. Schrek and Preston[2], using the Bagg rat lymphosarcoma in Sprague–Dawley rats, report the successful demonstration of serum cytotoxins in the rats (25 per cent of the total) in which the tumour had regressed.

The present communication deals with a limited investigation on the response of a human male to two cultures of human adenocarcinoma, arising in two different individuals.

Cultures of the neoplastic tissue were used instead of tissue homogenates in an effort to obtain as pure a cellular antigen as possible. Fresh cultures were used instead of known cultures of long standing because of the reported tendencies of long-term culture lines to become alike in antigenic characteristics[3].

(1) A healthy white male, blood type O,Rh-positive, was used as the antibody producer.

(2) Fragments of three adenocarcinomas were obtained from males at primary surgical removal and cultured by Puck's methods until pure cultures of the neoplastic cells were obtained. These separate cultures were labelled *C.P.*, *A.N.* and *C.G.*

(3) A portion of the living culture *C.P.*, estimated to be of the order of 10,000,000 cells, was suspended in salt solution and injected subcutaneously in the upper arm. The implant increased in size for ten days with marked local redness of the skin and moderate tenderness. By the fourteenth day the implant had decreased in size by 50 per cent. There was no systemic effect noted.

On the eighteenth day a similar amount of culture *C.P.* was introduced as before in a new location. The local reaction was immediate but decreased by half by the sixth day after implantation.

A third implantation of like amount was accompanied by local reaction more violent than the preceding two and by mild general systemic reaction lasting 24 hr. Disappearance of the local mass was almost complete at the end of the sixth day.

Seven days after the third implantation of *C.P.*-cultured cells, blood was withdrawn, the serum separated and an amount of this serum, equal to 1/400 of the total nutrient solution, was added to the nutrient solution consisting of 40 per cent adjunct nutrient solution. The nutrient solution, minus the immune serum addition, was the one used previously on all cell cultures and had proved adequate for growth.

A culture each of *C.P.*, *A.N.* and *C.G.* was drained of nutrient solution, washed and the nutrient solution containing the immune serum introduced.

The cultures were examined at 6, 12 and 24 hr. Culture *C.P.* at the end of 6 hr. showed nuclear degeneration, the nucleus in most cases forming an amorphous halo. There was marked rounding and shrinking of the cells. Subcultures taken at this time failed to grow, as did those made at 12 and 24 hr. Cultures *A.N.* and *C.G.* showed no effect of the immune serum, and both were successfully subcultured.

An identical series of implantations on the same individual were made except that in the second case cultures of *A.N.* were used as antigen. There were

Table 2. Adsorption Characteristics of Sweet and Tasteless Isomeric Nitroanilines

Compound	Wave-length for analysis	Molar absorbancy index
	(mμ)	
(I) 2-Bromo-5-nitroaniline (sweet)	247	9,840
(II) 4-Bromo-3-nitroaniline (tasteless)	237	16,320
(III) 2-Methoxy-5-nitroaniline (sweet)	253	12,320
(IV) 4-Methoxy-3-nitroaniline (tasteless)	228	16,900

Compound	Adsorption on charcoal			Adsorption on hair		
	α	$1/n$	W	α	$1/n$	W
I	0·340	0·830	0·0743	0·0436	0·417	0·0095
II	0·248	0·671	0·0538	0·0495	0·484	0·0108
III	0·269	0·732	0·0585	0·0244	0·494	0·0041
IV	0·186	0·745	0·0403	0·0125	0·275	0·0021

In the search for correlations between taste and molecular properties, a few properties which could affect the taste of a compound were studied first. Although some of these properties had been measured by previous investigators, the present work differs in that all measurements were made by the same group of investigators on compounds the tastes of which had been evaluated by the same panel. The results are given in Table 1.

Our second approach for finding a clue to the molecular properties responsible for characteristic tastes is to consider mechanisms by which sapid substances might trigger nerve impulses to the brain. Table 2 contains the results of a search for a correlation between the sweet taste and adsorption on a certain surface. Charcoal was used first in order to work out the experimental procedure. The hair was that of a child, washed in a nonionic detergent, and air-dried at room temperature. Two types of tongue tissue were used in unreported experiments but the results were not reproducible. The results in Table 2 fit the Freundlich equation :

$$x = \alpha c^{1/n}$$

where x represents millimoles of adsorbate held per unit weight of adsorbent ; c the concentration of adsorbate remaining unadsorbed at equilibrium in moles/litre ; α and n are constants ; while, in Table 2, W is the weight of substance adsorbed at the concentration of 0·01 gm./100 gm. solution.

Experiments were done to detect any special interaction between sweet compounds and certain biological substances. The results from one type of experiment are given in Table 3, from which no significant difference is observed between a sweet compound and its tasteless isomer. The numbers I and II refer to the compounds in Table 2. Other experiments were conducted to learn whether or not certain components common to enzyme systems are involved in taste chemireception. Thus, sweet solutions of 2-methoxy-5-nitroaniline and salty sodium chloride solutions were tasted in a helium atmosphere. Other than a sort of warming sensation, no significant difference in taste from that in air was observed. Sweet solutions of 2-methoxy-5-nitroaniline were tasted when diluted with solutions of magnesium chloride, manganous chloride, or water. The solutions containing magnesium or manganous ions were bitter and sweet, and magnesium ion could not be distinguished from manganous ion by taste. Then, to see whether or not the sapid substance interacts directly with an enzyme, solutions of a sweet compound were tasted in the presence of certain enzyme inhibitors. For example, several aliquots of a sweet solution of 2-methoxy-5-nitroaniline were diluted separately with water, or with solutions of sodium azide, potassium fluoride, sodium iodoacetate, or sodium cyanide. These solutions were mixed and tasted in random order several times without their identity being known to the taster (L. N. F.). Azide enhanced the sweetness of the nitroaniline, but fluoride and iodoacetate diminished the sweetness a very small amount, iodoacetate gave an iodine flavour, and cyanide was very bitter, outweighing the sweet taste. However, none of these recognized enzyme inhibitors obliterated the sweet taste irreversibly. This fact suggests that the taste receptor surface is not that of an enzyme.

Thus several physical properties which conceivably could be factors determining the tastes of substances have been measured. Also, various possible mechanisms for stimulus-receptor interactions have been examined. Although no positive generalizations have been found, and these experiments have been exploratory, the results serve as guides for future work in pursuing the identity of the molecular proper-

Table 3. Light Absorbancies of Various Solutions Alone and When Mixed

Compound	A^a	Sum	Change when mixed (per cent)	Compound	A^b	Sum	Change when mixed (per cent)
I	0·438			II	0·182		
Saliva	0·444	0·882		Saliva	0·200	0·382	
Mixture	0·845		−4·2	Mixture	0·360		−5·75
I	0·438			II	0·428		
Bovine gamma globulin	0·064	0·502		Bovine gamma globulin	0·135	0·563	
Mixture	0·486		−3·2	Mixture	0·534		−5·5
I	0·428			II	0·418		
ATP	0·145	0·573		ATP	0·078	0·496	
Mixture	0·563		−1·8	Mixture	0·478		−3·6
I	0·408			II	0·398		
ATP	0·112			ATP	0·048	0·446	
Mg^{2+}	0·000	0·520		II, ATP, Mg^{2+}	0·452		
Mixture	0·528		c. 0	II, ATP	0·452		c. 0
I	0·433			II	0·423		
Globin[c]	0·051	0·484		Globin	0·053	0·476	
Mixture	0·449		−7·8	Mixture	0·452		−5·3
Mixture + Mg^{2+}	0·452			Mixture + Mg^{2+}	0·454		

[a] Measurements made at the $\lambda_{max.}$ of I.
[b] Measurements made at the $\lambda_{max.}$ of II.
[c] A filtered colloidal solution of globin.
ATP, adenosine triphosphate.

ties responsible for the characteristic tastes of substances. It is to be realized that the nature of the stimulus-receptor interaction is unknown and these exploratory-type experiments constitute an attempt to find a clue on a gross level.

This work was undertaken in co-operation with the Quartermaster Food and Container Institute for the Armed Forces, Quartermaster Research and Engineering Command, U.S. Army.

AETIUS R. LAWRENCE
LLOYD N. FERGUSON

Chemistry Department,
Howard University,
Washington, D.C.

[1] *J. Chem. Educ.*, **35**, 436 (1958).

Energy Compensation in the Crabtree Effect with Ehrlich Ascites Carcinoma Cells

RECENTLY, Quastel and Bickis[1] published a very interesting and stimulating article concerning the energy relationships in several intact cell types, including the Ehrlich ascites carcinoma cell. They determined the ratio of lactate produced to the difference between oxygen consumed in the cell system with and without added glucose (average value 5·8). The assumption was made that the amount of adenosine triphosphate produced in the two systems was the same. They reasoned that if the total production of energy remained constant in the two cell systems, the ratio phosphorus/oxygen would be about 3.

Table 1. ENERGY RELATIONSHIP BETWEEN GLYCOLYSIS AND THE DEPRESSION OF RESPIRATION OF THE CRABTREE EFFECT

Compound (μM per ml. cells)	With glucose	Endogenous	Lactate / Δ Oxygen
Oxygen utilized	16·3	27·7	
Lactate accumulated	57·2	—	5·0
Adenosine triphosphate present	1·60	1·65	(P : O = 2·5)

Incubation system: 12·4 volume per cent of tumour cells in phosphate Locke's solution, *p*H 7·4; 30° C. for 15 min.; glucose 51·7 μM per ml. cells.

We recently published observations[2] on the Ehrlich ascites cells which show that the adenosine triphosphate content of the endogenous- and the glucose-containing cell is the same, except for an initial brief period of equilibration. This means that glycolysis does produce an amount of energy equivalent to the energy lost by the decreased oxygen consumption, provided the utilization of adenosine triphosphate remains constant. Furthermore, Table 1 shows that the accumulation of lactate could nearly account for the decrease in oxygen consumption in terms of production of adenosine triphosphate, assuming a phosphorus/oxygen ratio of approximately 3 (2·5) and glycolytic production of 2 molecules of adenosine triphosphate per glucose molecules. These results are in good agreement with Quastel and Bickis's hypothesis.

K. H. IBSEN
E. L. COE
R. W. MCKEE

Department of Physiological Chemistry,
University of California,
Los Angeles 24.

[1] Quastel, J. H., and Bickis, I. J., *Nature*, **183**, 281 (1959).
[2] Ibsen, K. H., Coe, E. L., and McKee, R. W., *Biochim. Biophys. Acta*, **30**, 384 (1958).

PATHOLOGY

Homologous Species Neoplastic Antibodies

Prehn and Main[1], in a review of the subject, state that the demonstration of circulating cytotoxins is much simpler when heterologous tissue transplants are used as antigens than when homologous tissues are used. Schrek and Preston[2], using the Bagg rat lymphosarcoma in Sprague–Dawley rats, report the successful demonstration of serum cytotoxins in the rats (25 per cent of the total) in which the tumour had regressed.

The present communication deals with a limited investigation on the response of a human male to two cultures of human adenocarcinoma, arising in two different individuals.

Cultures of the neoplastic tissue were used instead of tissue homogenates in an effort to obtain as pure a cellular antigen as possible. Fresh cultures were used instead of known cultures of long standing because of the reported tendencies of long-term culture lines to become alike in antigenic characteristics[3].

(1) A healthy white male, blood type O,Rh-positive, was used as the antibody producer.

(2) Fragments of three adenocarcinomas were obtained from males at primary surgical removal and cultured by Puck's methods until pure cultures of the neoplastic cells were obtained. These separate cultures were labelled *C.P.*, *A.N.* and *C.G.*

(3) A portion of the living culture *C.P.*, estimated to be of the order of 10,000,000 cells, was suspended in salt solution and injected subcutaneously in the upper arm. The implant increased in size for ten days with marked local redness of the skin and moderate tenderness. By the fourteenth day the implant had decreased in size by 50 per cent. There was no systemic effect noted.

On the eighteenth day a similar amount of culture *C.P.* was introduced as before in a new location. The local reaction was immediate but decreased by half by the sixth day after implantation.

A third implantation of like amount was accompanied by local reaction more violent than the preceding two and by mild general systemic reaction lasting 24 hr. Disappearance of the local mass was almost complete at the end of the sixth day.

Seven days after the third implantation of *C.P.*-cultured cells, blood was withdrawn, the serum separated and an amount of this serum, equal to 1/400 of the total nutrient solution, was added to the nutrient solution consisting of 40 per cent adjunct nutrient solution. The nutrient solution, minus the immune serum addition, was the one used previously on all cell cultures and had proved adequate for growth.

A culture each of *C.P.*, *A.N.* and *C.G.* was drained of nutrient solution, washed and the nutrient solution containing the immune serum introduced.

The cultures were examined at 6, 12 and 24 hr. Culture *C.P.* at the end of 6 hr. showed nuclear degeneration, the nucleus in most cases forming an amorphous halo. There was marked rounding and shrinking of the cells. Subcultures taken at this time failed to grow, as did those made at 12 and 24 hr. Cultures *A.N.* and *C.G.* showed no effect of the immune serum, and both were successfully subcultured.

An identical series of implantations on the same individual were made except that in the second case cultures of *A.N.* were used as antigen. There were

no major differences in reaction as compared to the first run.

The immune serum, used in the same concentration as before, now produced nuclear degeneration and death of cells in both culture *C.P.* and *A.N.* Culture *C.G.* remained unaffected by the immune serum–nutrient mixture, both morphologically and by successful subculture.

Heating the immune serum for 40 min. at 56° C. destroyed its cytotoxic activity, which was restored on the addition of fresh non-immune human serum.

Hoy A. Cranston
Frederick O. Eggert

Laboratory of Polarographic Analysis,
7002 South Halsted Street,
Chicago 21. Feb. 19.

[1] Prehn, R. T., and Main, J. M., *J. Nat. Cancer Inst.*, **14**, 537 (1953).
[2] Schrek, Robert, and Preston, Frederick W., Surgical Forum of the American College of Surgeons (1954).
[3] Coriell, Lewis L., Tall, Milton G., and Gaskill, Helen, *Science*, **128**, 198 (1958).

PSYCHOLOGY

The Water–Salt Receptor and Preference in the Rat

Zotterman[1] and his co-workers have reported that in the rat and in man the taste of water consists in a reduction of the spontaneous firing of the salt receptors. According to Deutsch's theory[2] of need and reward, satiation of thirst is due to the reception of a certain quantity of messages signalling the ingestion of water. The quantity of fluid ingested would be proportionate to the number of signals generated by it. It follows that as a hypotonic solution of saline reduces the spontaneous firing of salt receptors less than the same volume of water, it would generate less of a 'water signal'. A rat should therefore drink more saline solution even when water is present. This has already been noted by workers such as Richter[3] and Bare[4] and ascribed to a preference for weak saline solutions. But if the above hypothesis is correct, the ingestion of saline by the rat is not due to a preference but to the fact that it has been given 'diluted water', if we can put it that way.

On this theory a certain amount of reward is also proportionate to the total quantity of messages signalling the ingestion of a goal substance. Therefore if this hypothesis is correct, we should predict the opposite 'preference' in a situation where the intake of each fluid is restricted to a certain volume. Thus rats, when they are forced to choose between equal amount of water and saline in a maze, should learn to run to the water arm, as a given volume of saline generates fewer signals than the same volume of water. Accordingly, two experiments were carried out.

The first was carried out in a T-maze. Nine rats, aged 4 months, were given 2 hr. of *ad lib.* water drinking a day and *ad lib.* food except during maze running. Each animal was given 15 runs a day in the T-maze under 20 hr. of water deprivation to rewards of water in one arm and 0·5 per cent saline in the other, four animals receiving water in the right arm and five in the left. The average number of runs to water over the 6 days was 56, and to the saline, 34 (significant at the 0·001 level of probability).

In the second experiment, a group of 10 rats was put on the same schedule as those above. They were presented with two water bottles for half an hour when they were 20 hr. thirsty, one containing 0·5 per cent saline, the other water. The position of the bottles varied. The experiment confirmed that during eight days the average amount of saline drunk was 59 c.c. as against 36 c.c. (significant at the 0·01 level of probability).

The experiment therefore confirms the prediction and also casts considerable doubt on the conventional ways of measuring preference. It is hoped to investigate further the behaviour of species which lack this gustatory arrangement, and other parameters of the ingestion of saline by the rat. Further, the spontaneous rate of firing of the saline receptors should vary with the degree of salt depletion. This should in its turn reduce the level of water intake. Whether such a simple control mechanism does in fact govern the ingestion of water in relation to salt suggests a further line of investigation.

J. A. Deutsch
A. D. Jones

Institute of Experimental Psychology,
University of Oxford.

[1] Zotterman, Y., *Nature*, **183**, 191 (1959).
[2] Deutsch, J. A., *Brit. J. Psychol.*, **47**, 115 (1956); **44**, 304 (1953).
[3] Richter, C. P., *Amer. J. Physiol.*, **115**, 155 (1936).
[4] Bare, J. K., *J. Comp. Physiol. Psychol.*, **42**, 242 (1949).

PALÆONTOLOGY

Precambrian Coelenterata from Australia, Africa and England

Extensive collecting has been carried out during the past two years by private collectors, the South Australian Museum, the University of Adelaide and Mr. R. C. Sprigg and associates, in flaggy quartzites near the base of the Cambrian at the old Ediacara mines, 280 miles due north of Adelaide, South Australia. Sprigg[1] found and described as jellyfishes fossil remains of soft-bodied animals from this locality. The study of the abundant new material[2], which comprises about 800 specimens, has led to the following results. (1) The fauna consists not only of the forms described by Sprigg as scyphozoan and hydrozoan medusæ, but also of Anthozoa representing the Octocorallia, of Annelida[3], and of at least two entirely new types of soft-bodied invertebrates[2,3]. (2) Certainly one and possibly more elements of this fauna belong to genera described from the Kuibis Quartzite of the Nama System in South Africa, and one genus recently described[4] from the Precambrian of Charnwood Forest, Leicestershire, also occurs at Ediacara. (3) New stratigraphical and palæontological evidence indicates Late Precambrian age of the Ediacara fauna.

The most striking and unexpected discovery is the common occurrence of representatives of the South African genus *Rangea* Gürich[5] in the Ediacara fauna. About forty specimens are now known, and one new species has been described, though further finds may indicate the presence of more species in this material. It is preserved in different forms, which can be interpreted as different aspects and stages of preservation of the soft body. Some specimens show a long stalk attached to the leaf-shaped main body. This has either a median field or a median zig-zag furrow, and lateral furrows branching out from it. A spicular structure is seen occasionally in the body and stalk. The details of these structures, which will be described elsewhere[2], clearly establish a close relationship between *Rangea* and the living Pennatulacea In.

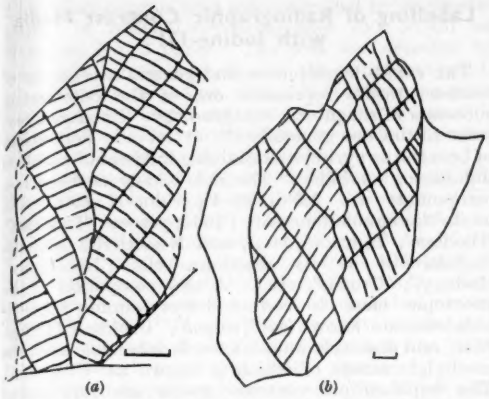


Fig. 1. *a*, Portion of the 'frond' of *Charnia masoni* Ford from the Precambrian of Charnwood Forest, Leicestershire (copy of portion of Ford's Pl. 13, Fig. 3). *b*, *Charnia* sp. from Ediacara, South Australia. S. Aust. Mus. No. P12897. Length of each scale 1 cm.

this order of the Anthozoa the polyps sit either on the rhachis or on discrete leaf-like lateral extensions; *Rangea* may be intermediate between these two groups as the lateral 'branches' are not free as in *Pennatula* but form a single leaf. The genus *Pteridinium* Gürich, which is common in the Kuibis Quartzite, is at present represented in the South Australian fauna only by two poorly preserved specimens. As Richter has observed, it belongs to the same family as *Rangea*; but this cannot be placed in the Gorgonacea with which it has no distinctive features in common, but belongs to the Pennatulacea. These had been previously reported from the Tertiary, Cretaceous and Jurassic and, with some doubt, from the Silurian and Ordovician.

The recent discovery[4] of fossils in the Charnian of Charnwood Forest is of extraordinary interest in this connexion. A single specimen from Ediacara, with the proximal and distal ends broken off (Fig. 1), was classified as *Rangea*(?) sp. before the description of *Charnia* Ford became available. There is no doubt that the two fossils are congeneric; but the South Australian specimen is almost twice the size of the holotype of *Charnia masoni* Ford. The author left the affinities of this fossil in doubt but concluded that it "may most rationally be interpreted as an algal frond. . . . The only likely alternative is that they [that is, *Charnia* and the associated *Charniodiscus*] represent a primitive coelenterate of unknown affinities". *Charnia* is sufficiently close to *Rangea* to be considered as related to the Pennatulacea.

This raises the question of the nature of *Charniodiscus*. Ford has suggested a possible relationship with *Charnia*, with the medusoid disk-like fossil forming the base of the stalk to which the frond-like *Charnia* is attached at the other end. A similar relationship between some of the medusoid fossils from Ediacara from which stalk-like bands emerge and the stalked *Rangea* has been considered, but the two have not yet been found actually joined in one specimen. The presence of a disk-shaped base would distinguish *Rangea* and *Charnia* from all living Pennatulacea in which the end of the stalk is merely club-shaped; but in my opinion it would not exclude close relationship. The presence of a medusa-like base which would correspond to the primary axial polyp of a Pennatulacean could lead to phylogenetic speculations about the still obscure relationships of Scyphozoa and Anthozoa. Such speculation would be premature; but it should be kept in mind when the medusoid Precambrian fossils are re-examined.

The Ediacara fauna is placed in the Late Precambrian for the following reasons. It is found 100–200 ft. below the top of a widespread thick quartzite formation (Pound Quartzite) which is conformably overlain by about 500 ft. of limestones with *Archaeocyatha* and brachiopods in the uppermost beds. This assemblage No. 2 of Daily[6] is widespread in South Australia and can be confidently placed low in the Lower Cambrian. Boulders of Archæocyatha limestones with this and successive assemblages Nos. 3 and 4 occur below shales with Late Lower Cambrian protolenids and *Redlichia* on Kangaroo Island. The Ediacara fauna, taken from a horizon below an early Lower Cambrian fauna, is unlike any known Cambrian fossil assemblage. It is therefore correctly assigned to the Late Precambrian. The same age can now be suggested on palæontological grounds for the Kuibis Quartzite of the Nama Group and for the fossiliferous strata of the Charnian.

M. F. Glaessner

University of Adelaide,
South Australia. Feb. 27.

[1] Sprigg, R. C., *Trans. Roy. Soc. S. Aust.*, **71**, 212 (1947); **73**, 72 (1949).
[2] Glaessner, M. F., and Daily, B., *Rec. S. Aust. Mus.*, **13**, Part 3 (in the press).
[3] Glaessner, M. F., *Trans. Roy. Soc. S. Aust.*, **81**, 185 (1958).
[4] Ford, T. D., *Proc. Yorks. Geol. Soc.*, **31**, 211 (1958).
[5] Richter, R., *Senckenb. Leth.*, **36**, 243 (1955).
[6] Daily, B., Twentieth Int. Geol. Congr. El Sist. Cambr., **2** (2), 91 (1956).

CHEMISTRY

Some Alkyl-Aryl Sodium Sulphonates as Standard Substances in Detergent Chemistry

In the course of work on the behaviour of commercial detergents of the anionic type, we needed some standard substances with which to compare the commercial products. The sodium sulphonates of the straight-chain substituted benzene hydrocarbons appeared to be likely compounds. The required hydrocarbons from butyl benzene to tetradecyl benzene were synthesized from the fatty acids with even numbers of carbon atoms. The branched-chain compound α-dimethyldecyl benzene was also synthesized. From the hydrocarbons the sodium sulphonates were prepared and purified. The behaviour of these compounds in solution was studied by means of the methylene blue method of Longwell and Maniece[1] and by paper chromatography.

Methylene blue tests on pure sodium sulphonates of the hydrocarbons. Standard solutions of the pure sodium sulphonates of the hydrocarbons were prepared by weighing known amounts of the pure dry substances and dissolving in water to standard volumes. There is a decrease in solubility with increasing chain-length, and special care is required to obtain solutions of the higher members at a suitable concentration. As an example, the solubility of sodium-tetradecylbenzenesulphonate is 6·5 parts per million at 20° C. Aliquots of these solutions were examined by the methylene blue method, and the optical densities of the resulting coloured chloroform extracts were recorded. After allowing for reagent blanks, which were run with each experiment, plots were made of optical density against concentration and from the graphs so obtained Table 1 was derived. From column 4 of Table 1 it is seen that optical

Table 1

Substance	Molecular weight	Value of optical density 200 μgm. of sulphonate	Optical density × molecular weight	
n-Tetradecylbenzenesulphonate	376	0·935	352	
n-Dodecylbenzenesulphonate	348	1·010	351	Average value 347
n-Decylbenzenesulphonate	320	1·075	344	
n-Octylbenzenesulphonate	292	1·166	340	
n-Hexylbenzenesulphonate	264	1·138	300	86% of av.
n-Butylbenzenesulphonate	236	0·106	22	6% ,,
α-Dimethyldecylbenzenesulphonate	346	0·926	320	93% ,,
Alkylbenzenesulphonate of commerce	356	0·926	330	96% ,,
'Manoxol O.T.'	444	0·780	346	100% ,,

density multiplied by the molecular weight is approximately a constant for the members of the series from *n*-octylbenzene upwards. This is a useful check on the molecular weight of an unknown compound of similar composition. Figures for α-dimethyldecylbenzene sodium sulphonate, the alkylbenzene sodium sulphonate of commerce and for manoxol (sodium-dioctylsulphosuccinate) are included for purposes of comparison. The sodium sulphonates below butyl benzene in the series give no colour under the conditions of the Longwell–Maniece test.

Paper chromatography of some alkyl-aryl sodium sulphonates. Commercial detergents of the alkyl-aryl sulphonate type are difficult to study by paper chromatography, and special methods such as that of F. Franks[2] have been devised for their examination. The difficulties appear to be due to their detergent properties which cause them to remove other substances, including other detergents, from the paper and to carry them forward with their own movement. These remarks also apply to α-dimethyldecylbenzenesodium sulphonate and to detergents of the 'Teepol' type. With the straight-chain alkyl-aryl sulphonates there are no such effects and chromatography proceeds normally. Values of R_F for four such compounds in various mixtures of alcohol and water are given in Table 2. Whatman No. 4 paper was used and the spots were shown up with a solution of rhodamine 6*B* used as a spray at a concentration of 0·05 per cent w/v.

Table 2. R_F Values

Substance	Per cent alcohol/water by volume					
	10	30	35	40	50	100
n-Octylbenzenesulphonate	0·67	0·81	0·82	0·83	0·87	0·89
n-Decylbenzenesulphonate	0·03	0·54	0·74	0·75	0·84	0·89
n-Dodecylbenzenesulphonate	Nil	0·22	0·23	0·23	0·76	0·89
n-Tetradecylbenzenesulphonate	Nil	0·06	0·06	0·10	0·20	0·89

We wish to thank the Government Chemist for permission to publish this communication.

E. Q. Laws
W. Hancock
Department of the Government Chemist,
Government Laboratory,
Clement's Inn Passage,
London, W.C.2. April 3.

[1] Longwell, J., and Maniece, W. D., *Analyst*, **80**, 167 (1955).
Franks, F., *Nature*, **176**, 603 (1955).

Labelling of Radiographic Contrast Media with Iodine-131

The rapid development and general acceptance of contrast media represents one of the outstanding successes of diagnostic röntgenology. Roentgen contrast media are generally di- or tri-iodo derivatives of benzene or pyridine, containing further substituents influencing solubility. The most widely used contrast compounds are 3,5-diiodo-4-pyridon-N-acetic acid as its diethanolamine salt ('Iodopyracet', 'Diodrast', 'Diodone', 'Ioduron', etc.), and N-methyl-3,5-diiodochelidamate as its disodium salt ('Neo-Iopax', 'Iodoxyl', 'Uropac', etc.). A new compound of the urotropic class is sodium-3-acetyl-amino-2,4,6-triiodobenzoate known as 'Triopac', 'Urokon', 'Triumbren' and diacetylamino-2,4,6-triiodobenzoic acid, the methylglucamate of which is known as 'Urografin'. The hepatotropic contrast media are represented by N,N′-adipyl-*bis*(3-amino-2,4,6-triiodo)-benzoic acid as methylglucamate ('Biligrafin' or 'Cholografin') and 2-ethyl-3-(3′-amino-2,4,6-triiodophenyl)-propionic acid ('Cistobil', 'Telepaque').

The application of these contrast media for diagnostic purposes gives only a static picture. Pathological changes are diagnosed on the basis of permanent differences in the morphological picture. It is not generally possible to gain information on the functional state of the organs by these means.

The labelling of contrast compounds with iodine-131 opens the possibility of investigating the functional state of the organs by measuring the amount of the compound applied and excreted and its concentration. Indicator diagnosis with the aid of labelled contrast media allows the registration and analysis of the dynamics of processes, and therefore combines both possibilities.

In this communication a method for labelling the more important contrast media with iodine-131 is presented; these are 3,5-diiodo-4-pyridon-N-acetic acid ('Ioduron', 'Diodon'), 3-acetylamino-2,4,6-triiodobenzoic acid ('Triopac', 'Triumbren'), N,N′-adipyl-*bis*(3-amino-2,4,6-triiodo)-benzoic acid ('Biligrafin') and 3,5-diacetylamino-2,4,6-triiodobenzoic acid ('Urografin'). An exchange reaction method was used. The use of labelled contrast media ('Triopac') for determining the vitality of bone tissue will be described elsewhere.

Sodium-3-acetylamino-2,4,6-triiodobenzoate-^{131}I, the preparation used, was 'Triopac-400' (Cilag A.G., Schaffhausen, Switzerland) or 'Triumbren' (Spofa, Prague); 2 ml. of the solution contains 1·292 gm. of the sodium salt.

To 2 ml. of 'Triopac-400' solution were added 8 ml. acetate buffer solution *p*H 5, 1 ml. potassium iodide carrier solution (20 mgm. in 100 ml. water) and 4 ml. potassium iodide-131 solution with a radioactivity of 9 mc. The mixture was heated under reflux on the water-bath for 12 hr. The solution was then allowed to cool to room temperature and free 3-acetylamino-2,4,6-triiodobenzoic acid precipitated by addition of concentrated phosphoric acid to *p*H 1. The precipitate was filtered by suction and washed with cold water until the volume of the filtrate was exactly 32 ml. The loss of radioactivity amounted to 1·5 per cent. The precipitate was dissolved in a slight excess of ammonia and the precipitation repeated twice (yield, 1·12 gm. of the free acid (89 per cent) and 90 per cent of the original radioactivity). The purity of the 3-acetylamino-

2,4,6-triiodobenzoic acid was controlled by chromatography in butanol/ammonia/water (50 : 5 : 45) on Whatman No. 3 paper. The acid was detected by radioactivity measurement, and the potassium iodide by oxidation to iodine with 0·1 *N* ferrous nitrate solution containing 3 per cent of hydrogen peroxide and starch. The recrystallized products were not contaminated by potassium iodide.

3,5-Diacetylamino-2,4,6-triiodobenzoic acid-^{131}I, 'Urografin' solution 76 per cent (Schering A.G., Berlin), containing 2 gm. of the sodium and 13·2 gm of the methyl-glucamine salt in 20 ml. (2 ml. of the 'Urografin' solution correspond to 1·193 gm. of 3,5-diacetylamino-2,4,6-triiodobenzoic acid) were iodinated by the exchange reaction described above. The yield was 0·775 gm. (65 per cent) of the acid and 66·4 per cent of the original radioactivity. The product was chromatographically pure.

3,5-Diiodo-4-pyridon-N-acetic acid-^{131}I, 70 per cent 'Ioduron' (Cilag A.G.) or 'Diodon' (Spofa) was employed as the diethanolamine salt. 2 ml. 70 per cent 'Ioduron', corresponding to 1·11 gm. of the free acid, was labelled as described above. The yield was 67 per cent of the acid and 70 per cent of the original radioactivity. The product was chromatographically pure.

5 ml. of a 30 per cent solution of the methyl-glucamine salt of 3,3′-(adipyl-diimino)*bis*-2,4,5-triiodobenzoic acid 'Biligrafin' (Schering A.G., Berlin) containing 1·125 gm. of the free acid was labelled as above. The yield was 45 per cent of the acid and 46·5 per cent of the original radioactivity. The product was chromatographically pure. Identical yields were obtained with the corresponding Spofa product.

The radioactivity of the samples was determined in liquid samples with the aid of a scintillation counter.

J. Liebster
J. Kácl
A. Babický

Radioisotope Laboratories,
Biological Institute,
Czechoslovak Academy of Science and
Radiological Clinic of the University,
Prague.
March 3.

Infra-red and the Near Ultra-violet Absorption Spectra of Polypheny. Derivatives of the Elements of Groups IV*b* and V*b*

We have been investigating the infra-red and the near ultra-violet absorption spectra of several polyphenyl derivatives of the elements of groups IV*b* and V*b*. In the case of the derivatives of the V*b* elements, we have studied the spectra of derivatives where the central atoms possess and do not possess unshared *p*-electrons (derivatives of the atoms in their 'trivalent' and 'pentavalent' states respectively). In addition, we have been studying the spectra of *o*-, *m*- and *p*-substituted tritylbenzenes (trityl = triphenylmethyl). The ultra-violet spectra were recorded in alcohol solutions, and the infra-red spectra in mulls and also in solutions.

Our results on the ultra-violet spectra of the triphenyl derivatives of the V*b* elements ($M\,Ph_3$ where M = V*b* element) agree well with those of Jaffe[1] and show trends similar to the derivatives of the type $Ph\,M\,Me_2$ studied by Bowden and Braude[2]. The ultra-violet spectra of the triphenyl and tetraphenyl halides ($Ph_n M\,X_m$ where X = Cl or Br and $n + m = 5$) are similar to those of the other derivatives of the pentavalent atoms[1–4]. In general, the phenyl derivatives of the V*b* elements in the trivalent state exhibit only one strong absorption band ($n \rightarrow \pi^*$) while the pentavalent atom compounds show vibrational structure ($\pi \rightarrow \pi^*$) similar to the secondary band (*B*-band at 2500 A.) of benzene itself. This indicates that the unshared *p*-electrons in the trivalent atom derivatives participate in resonance interaction with the π-electrons of the benzene system. This is further demonstrated by the progressive bathochromic shifts observed with the increase in the number of phenyl groups on the central atom. Such variations in spectra are considered to be proportional to the magnitude of the resonance interaction of the phenyl groups with the central atom[5]. The positions of the secondary bands of aniline, diphenylamine and triphenylamine are found to be 2800, 2850 and 2970 A. respectively. The primary band (*E*-band) seems to vary little, having the values of 2300 and 2280 A. for aniline and triphenylamine. Jaffe[1] did not seem to observe such variations in the series $PhNMe_2$, Ph_2NMe and NPh_3 because of the bathochromic shifts due to the methyl groups. He, however, obtained a progressive increase in the primary band wave-length with the number of the methyl groups. It is interesting to see that in spite of the bathochromic shift caused by the methyl groups, PPh_3 absorbs around 2610 A. compared with 2510 A. in the case of $Ph\,PMe_2$. The intensity is also considerably greater in the former compound. The arsenic and antimony derivatives also show similar variations.

Among the $M\,Ph_3$ derivatives of the V*b* elements, NPh_3 shows the largest hyperchromic effect, which seems reasonable, nitrogen being a first-row element. There is a marked decrease in the wave-length and the intensity in the case of PPh_3 and a further decrease in the wave-length in $AsPh_3$. While in phosphorus the 3*d* shell is vacant, in arsenic it is completely filled. But while going from $AsPh_3$ to $SbPh_3$ and $BiPh_3$ there are progressive shifts to longer wave-lengths (2480, 2560 and 2800 A.). This is considered to be due to the predominating influence of the decreasing ionization potential of the central atom. The importance of the ionization potentials in determining such bathochromic shifts has been recognized[6,7]. $BiPh_3$ also shows another shoulder around 2500 A., which may represent dissociation.

The presence of the vibrational structure of benzene in the derivatives of the pentavalent V*b* atoms indicates negligible or no resonance interaction of the central atoms with the π-electrons of the benzene system. The spectra of these derivatives show little variation with the number of phenyl groups attached to the atom. For example, both tetraphenylstiboniumchloride and triphenylstiboniumdichloride show the absorption maxima at 2700, 2630, 2580 and 2530 A. The derivatives of IV*b* elements behave similarly and show almost no variation in their spectra with the number of phenyl groups. Our results on the phenyl derivatives of IV*b* elements agree with those of Milazzo[8] and of Bowden and Braude[2] on derivatives of the type $PhMR_2$ (R = alkyl group). Among IV*b* derivatives, the silicon

derivatives seem to show slightly larger bathochromic shifts, while among the V*b* pentavalent atom compounds, the phosphorus derivatives show larger bathochromic shifts. This may possibly be taken as evidence for the expansion of the valence shell in these two second-row elements.

The ultra-violet spectra of the *o*-, *m*- and *p*-substituted tritylbenzenes also show the vibrational structure of benzene. There is little difference between the spectra of the *p*- and the corresponding *m*-derivatives. The *o*-derivatives seem to exhibit considerable steric interaction. Some of the *p*-substituents which have characteristic absorptions in the ultra-violet, exhibit their absorption maxima at the same wave-lengths as they would in the mono-substituted benzenes. There seems to be little doubt that the *p*-trityl group does not interact with the other substituent or with the benzene ring and that the resonance parameter of the trityl group is approximately zero. Further, the overall electronegativities of the *m*- and *p*-trityl groups were both estimated to be about zero, by a study of the characteristic group frequencies of the *m*- and *p*-derivatives in the infra-red. These conclusions are in accordance with the Hammett reactivity constants of the *m*- and *p*-trityl groups, which have been found to be close to zero by Benkeser and Gosnell[9].

All the polyphenyl derivatives of IV*b* and V*b* elements show mass effects (decrease in frequency with increase in mass) in their infra-red spectra. The mass effects are marked in the C=C skeletal vibrations, in some of the frequencies due to the mono-substituted phenyl group and in one of the C—H out-of-plane deformation bands (750 cm.$^{-1}$). The 750 cm.$^{-1}$ C—H out-of-plane deformation bands of these derivatives show unusual splittings. All these derivatives exhibit bands in the regions 1,440–1,400 cm.$^{-1}$ and 1,100–1,000 cm.$^{-1}$, indicating that such frequencies are by no means unique to P *Ph* or Si *Ph* groups[10]. They might as well be due to some activated ring vibrations.

Further work on these systems is in progress and a detailed report will be published elsewhere at a later date. We wish to thank Prof. R. A. Benkeser of Purdue University for kindly providing the pure samples of the tritylbenzenes.

C. N. R. Rao
J. Ramachandran
M. S. C. Iah*
S. Somasekhara†
T. V. Rajakumar‡

Department of Chemistry,
University of California,
Berkeley 4.
March 6.

Present addresses: * Karnatak University, Dharwar, India. † Northwestern University, Evanston, Illinois. ‡ University of Illinois, School of Medicine, Chicago.

[1] Jaffe, H. H., *J. Chem. Phys.*, **22**, 1430 (1954).
[2] Bowden, K., and Braude, E. A., *J. Chem. Soc.*, 1068 (1952).
[3] Mann, F. G., Millar, I. T., and Smith, B. B., *J. Chem. Soc.*, 1130 (1953).
[4] Jaffe, H. H., and Freedman, L. D., *J. Amer. Chem. Soc.*, **74**, 1069 (1952).
[5] Robertson, W. W., and Matsen, F. A., *J. Amer. Chem. Soc.*, **72**, 5250 (1950).
[6] Sklar, A. L., *J. Chem. Phys.*, **7**, 984 (1939).
[7] Matsen, F. A., *et al.*, *J. Amer. Chem. Soc.*, **72**, 5243, 5248, 5252 (1950).
[8] Milazzo, G., *Gazz. Chim. Ital.*, **71**, 73 (1941).
[9] Benkeser, R. A., and Gosnell, R. B., *J. Org. Chem.*, **22**, 327 (1957).
[10] Bellamy, L. J., "The Infrared Spectra of Complex Molecules" (Methuen, London, 1958).

Binding of Water in Ferric Hydrate

A recent communication discussed the infra-red spectra and proton magnetic resonance experiments on some hydroxides and hydrous oxides[1]. We found in the so-called ferric hydrate OH-groups beside free water (adsorbed), that is to say, this compound may be defined as iron(III) hydroxide. In my own communication, the drawing for the infra-red spectrum (Fig. 1) was incorrect; the centre of gravity of curve I should be between 3·1 and 3·2μ.

In Table 1 herewith, there are the bands of another precipitate with 19·9 per cent water. This was produced from a solution of ferric nitrate with ammonia.

Table 1

Infra-red spectrum of iron(III) hydroxide

Bands in cm.$^{-1}$ (br = broad)	I 3,300 br	II 1,625 br	III 1,030 br
Assignment	OH stretching vibration	H_2O bending vibration	OH bending vibration

From the infra-red spectra of the different iron hydroxides[2], the observed band III is assigned as a bending vibration of the OH-group. The possibility of a combination vibration of the adsorbed water can be excluded. Sometimes this band is split into two. It is possible to calculate[3] the Fe–OH distance with the wave-numbers of the bands I and III as ~ 1·90 A. This value is of the same order of magnitude as the corresponding distance in the other iron hydroxides (namely, γ-FeOOH, 1·97 A.), and hence the broadening of the bands and the amorphous structure of the compound prejudice the precision of the estimation. The infra-red spectrum of Table 1 may therefore be interpreted as showing that there are OH-groups besides water (adsorbed) in the compound under investigation.

O. Glemser

Anorganisch-chemisches Institut
der Universität,
Göttingen.

[1] Glemser, O., *Nature*, **183**, 943 (1959). Kroon, D., and v. d. Stolpe, C., *Nature*, **183**, 944 (1959).
[2] Glemser, O., and Hartert, E., *Z. anorg. allg. Chem.*, **283**, 111 (1956).
[3] Hartert, E., and Glemser, O., *Z. Elektrochem. angew. Physik. Chem.*, **60**, 746 (1956).

PHYSICAL SCIENCES

Geophysical Effects of High-Altitude Nuclear Explosions

Prominence has been given recently to some geophysical effects of the nuclear tests carried out in August 1958 at Johnston Island in the Pacific. According to newspaper reports[1], these tests included high-altitude explosions at heights of the order of 100 miles on August 1 and August 12; for each date the approximate time of the explosion was 1050 U.T. Observations[2] at Apia, Samoa, show that the test on August 1 coincided with the appearance of an aurora and the occurrence of radio fade-outs at Apia. The special significance of these phenomena is that Apia and Johnston Island lie approximately at opposite ends of a geomagnetic line of force, and the observations at Samoa have been explained[3] as due to the generation of charged particles by the test at Johnston Island and the subsequent guidance of the particles along the line of force to produce increased ionization in the lower ionosphere at Apia,

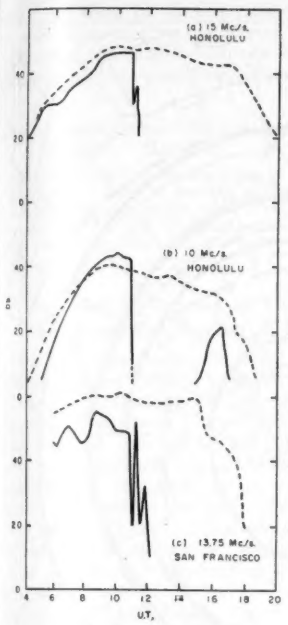


Fig. 1

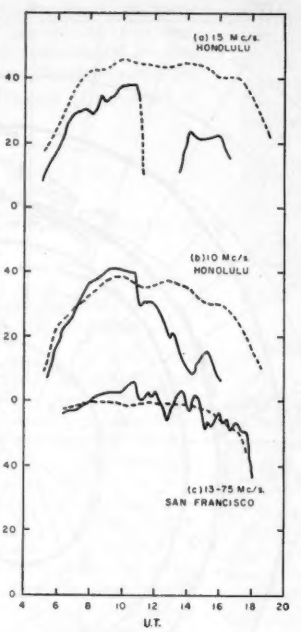


Fig. 2

Fig. 1. Variation with time of signals received at Hiraiso, Japan, on August 1, 1958

Fig. 2. Variation with time of signals received at Hiraiso, Japan, on August 12, 1958

the conjugate point. This mechanism would suggest that any effects are largely restricted to the vicinities of the two ends of the line of force; we wish to present evidence, however, strongly suggesting that increased *D*-layer ionization occurs for distances far greater from the explosion than has hitherto been realized, and at places remote from either the explosion or its geomagnetic conjugate.

Fig. 1 shows the diurnal variation in the strengths of three transmitters as received at the Hiraiso Radio Wave Observatory in Japan; the continuous curve is the record for August 1 and the dotted curve the expected diurnal variation. It may be noted that the time of the explosion coincides with a loss of signal on all three transmitters. The path from Honolulu to Hiraiso passes fairly close to Johnston Island, so that the effects in Figs. 1*a* and *b* are not unexpected. However, the path from San Francisco to Japan is some 3,600 km. from Johnston Island at its nearest point, and, of course, even more remote from the geomagnetic conjugate of Johnston. The records for August 12 are shown in Fig. 2. Again there is a pronounced effect for the Honolulu transmitter on 15 Mc./s. coinciding with the reported time of the explosion, but that on the 10 Mc./s. transmitter is less marked than on August 1, while no significant decrease is detectable for the San Francisco station.

An interesting record is shown on Fig. 3. This is of the integrated level of atmospherics on 28 kc./s. as received at Hiraiso on August 12; an enhancement at the time of the explosion is detectable. The coincidence in time almost certainly implies that the enhancement is due to the nuclear explosion, but there are unusual features. For example, Fig. 3 is dissimilar to the mean diurnal variation of noise on 27 kc./s. shown in Fig. 4, which is derived from Japanese data[4] for August 1957. Normally, one would anticipate the main sources of the atmospherics received in Japan at about 1100 U.T. in August to be in south-eastern Asia and India, with perhaps a subsidiary source, due to the secondary maximum of thunderstorm activity at local dawn, in South America. The enhancement on Fig. 3 could be interpreted as meaning that the source in South America is dominant; that the path of the atmospherics consequently lies close to Johnston; and that the signals are therefore greatly affected. Alternatively, the influence of the explosion may be so widespread as to affect the atmospherics from the thunderstorms in the East Indies, some 7,000 km. from Johnston. A solar flare with its associated sudden ionospheric disturbance could also produce the effect in Fig. 3; there are no reports, however, of such solar activity. Another possibility is a local thunderstorm, but again there are no records of any such storm; incidentally, the 28 kc./s. record for August 1 was completely dominated by thundery activity within Japan. Finally, recent talks at Geneva have shown that the explosion itself can be responsible for the generation of appreciable electromagnetic energy at low frequencies. Many of these alternatives could, of course, be eliminated if conventional wave-form and frequency spectra records for atmospherics were available, particularly if these were coupled with direction-finding and locating systems.

The effects discussed, namely, fade-outs on frequencies of about 10–20 Mc./s. and an enhancement of atmospherics on 28 kc./s., are entirely similar to those accompanying sudden ionospheric disturbance due to a solar flare. The disturbances are known to be caused by the production by the flare of increased

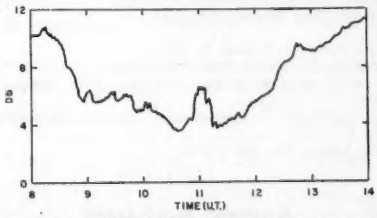


Fig. 3. Variation of atmospheric noise on 28 kc./s. for Hiraiso, Japan, on August 12, 1958

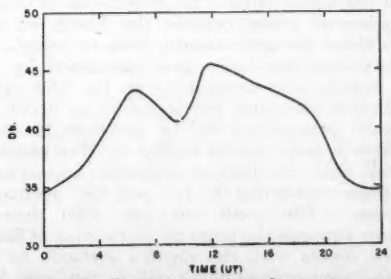


Fig. 4. Mean diurnal variation of atmospheric noise on 27 kc./s. for Japan in August

ionization in the ionospheric *D*-layer. It would appear probable that extra ionization is also produced in the same region by a high-altitude nuclear explosion, perhaps particularly when the explosion lies within the *D*-layer itself (height 70–100 km.). The record of Fig. 1c shows conclusively that the effects are not confined to regions influenced by particles guided by the Earth's magnetic field, the mechanism postulated in connexion with the ionization at the geomagnetic conjugate; the addition, incidentally, of precession along a geomagnetic line of latitude[3,5] still implies a considerable restriction in the areas affected. Other mechanisms must therefore also be invoked. Possibilities include analogies with the reported effects of thunderstorms on the ionosphere, including, for example, the direct production of ionization by the electromagnetic radiation of long wave-length generated by the explosion. Again, the production of shock waves with consequent magneto-hydrodynamic influences is feasible; in this connexion the violent oscillations of the record of Fig. 1c are intriguing.

T. Obayashi
Hiraiso Radio Wave Observatory,
Radio Research Laboratory,
Nakimato-shi, Ibaraki-ken.

S. C. Coroniti
E. T. Pierce
AVCO Research and Advanced Development Division,
Wilmington, Massachusetts.

[1] *New York Times*, 1, 5 (Aug. 2, 1958).
[2] Cullington, A. L., *Nature*, **182**, 1365 (1958).
[3] Kellogg, P. J., Ney, E. P., and Winckler, J. R., *Nature*, **183**, 358 (1959).
[4] Research Institute of Atmospherics, Nagoya University, I.G.Y. Data, **1** (1957).
[5] Gold, T., *Nature*, **183**, 355 (1959).

Auroral Isochasms

Theoretical reasons exist for believing that the auroral isochasms should be projections of circles in the equatorial plane outside the Earth on to its surface along the geomagnetic lines of force[1].

Such projections have been calculated by Hultqvist[2], taking into account up to the fifth term of the spherical harmonic development for epoch 1945. Four such projections on the northern hemisphere are drawn as solid curves in Fig. 1. The projections are ovals with the longest diameter approximately in the plane containing the 170° and 350° geomagnetic meridians. The ovals converge with decreasing diameters towards the point of intersection of Bartels's eccentric dipole with the Earth's surface[3], to which point the geomagnetic north pole is displaced by the higher terms of the harmonic development.

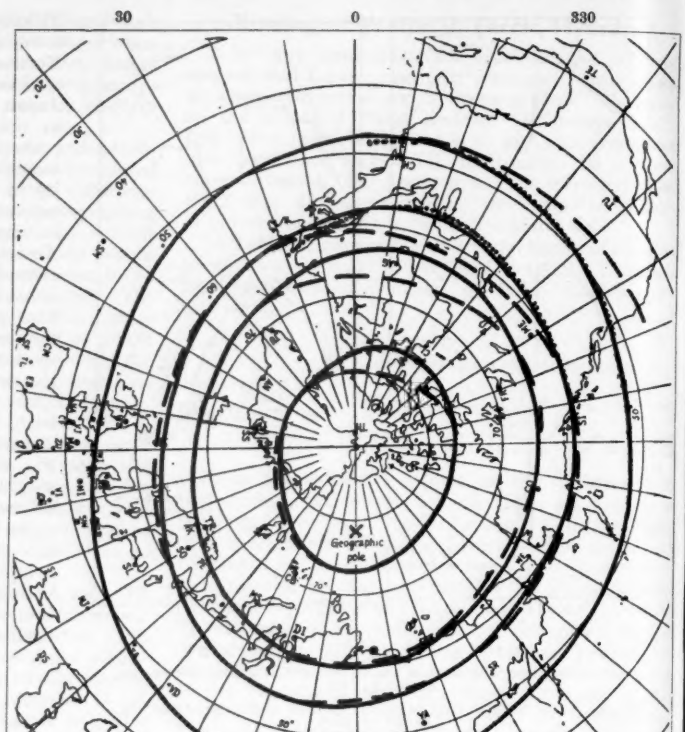


Fig. 1

Some of Vestine's[3] observational isochasms, which closely coincide with the calculated projections over most of the 360° of longitude, are also shown in Fig. 1 (broken lines). It can be seen that for the broken curves the agreement with the theoretical (solid) curves is good except in the region of north-eastern America, where the Vestine curves run up to some 4° too far to the north. The disagreement is so large and so systematic that, on the basis of the curves mentioned, the hypothesis of the isochasms being projections of circles in the equatorial plane seems somewhat doubtful.

Gartlein[4] has recently prepared new isochasms for the territory of the United States based on visual observations made during the International Geophysical Year. Two of those isochasms can be seen in Fig. 1 (dotted lines). They concern just those regions where Vestine's and the calculated isochasms disagree. It is interesting to see that Gartlein's isochasms differ considerably from Vestine's and agree very well with the theoretical curves. Only in the extreme eastern part—chiefly over the sea—do they appear a little too much to the north. The agreement is surprisingly good, especially for Gartlein's southernmost curve, if the uncertainty inherent in the method of evaluation of the observational curves is taken into account.

Thus the agreement between observed isochasms and projections of circles in the equatorial plane

along the lines of force seems to be good, in view of the uncertainty mentioned above of the experimental isochasms definitely demonstrated by the disagreement between Vestine's and Gartlein's curves.

BENGT HULTQVIST

Kiruna Geophysical Observatory,
Sweden. March 17.

[1] Alfvén, H., "Cosmical Electrodynamics" (Oxford, 1950).
[2] Hultqvist, B., *Arkiv för geofysik*, **3**, 63 (1958).
[3] Vestine, E. H., *Terr. Magn.*, **49**, 77 (1944).
[4] Gartlein, C. W., U.S. Visual Observations, News Letter No. 18 (Ithaca, New York, Feb. 23, 1959).

Possible Causes of Geomagnetic Fluctuations having a 6-Sec. Period

WE have delayed comment on F. B. Daniels's letter in *Nature*[1] hoping that we would obtain further information relevant to his suggestion that the fluctuations of about 6 sec. period in the geomagnetic field may be due either to microseisms or microbaroms. Although the general object of our main investigation has been to get a clear idea of the cause and origin of the geomagnetic fluctuations in a wide frequency-range, the main emphasis has been in the normal 'daytime' *pulsations continues* of 10–40 sec. period. The particular record referred to by Dr. Daniels[2] was taken during a magnetic storm, when geomagnetic fluctuations cover a range of periods from fractions of a second up to many minutes.

We have, however, observed that fluctuations in the range of 1–7 sec. period do sometimes appear on our records of $\delta X/\delta t$, $\delta Y/\delta t$ and $\delta Z/\delta t$ nearly simultaneously at two sites some hundreds of miles apart.

We have not attempted to measure microbaroms and would certainly encourage an investigation such as Dr. Daniels suggests, since it is possible that microbaroms are associated with some types of geomagnetic fluctuations. For example, pressure changes in the ionosphere may result from the influx of particulate charged material.

Recently, we have been paying more attention to fluctuations during the hours of darkness and especially during the magnetically quiet time of night. At this time, geomagnetic fluctuations may be associated with particulate matter from the Sun but probably not with direct radiation. We have noted a number of occasions when a characteristic damped fluctuation of about 1–3 min. period and maximum amplitude of $\frac{1}{2}$–2 gamma is readily related to a similar phenomenon simultaneously recorded some hundreds of miles away. However, the most interesting feature is that fluctuations of this type are sometimes associated with oscillations of periods of 2–7 sec. Rarely, these precede the main phenomenon by a minute or so, but more often they accompany it, and may continue for some time if the general magnetic activity is maintained. We believe the short and long periods are associated and both of electromagnetic origin.

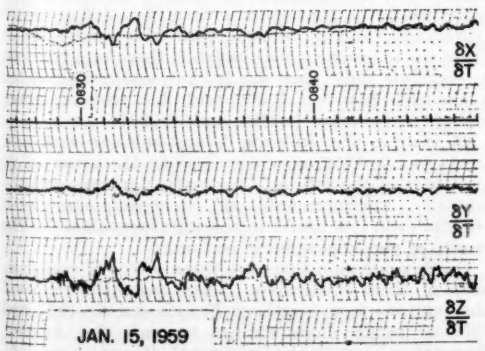


Fig. 1. Records of January 15, 1959, between 0812 and 0849 G.M.T. of $\delta X/\delta t$, $\delta Y/\delta t$ and $\delta Z/\delta t$ taken at Albert Head base station near Victoria, B.C.

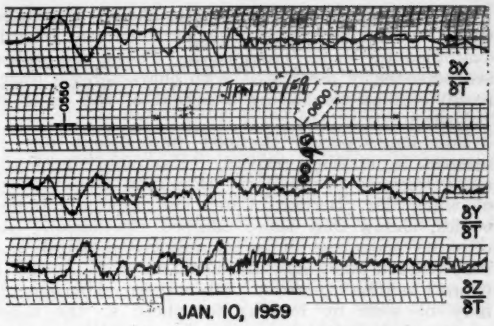


Fig. 2. Records of January 10, 1959, between 0544 and 0610 G.M.T. of $\delta X/\delta t$, $\delta Y/\delta t$ and $\delta Z/\delta t$ taken at Albert Head base station near Victoria, B.C.

Two dates which show the short-period oscillations starting just before the main train are 0819·7 on January 15, 1959, and about 0548·0 on January 10, 1959, G.M.T. We have been informed by Prof. Hugo Benioff that his seismograph records at the California Institute of Technology do not exhibit oscillations starting or increasing at the times and on the days mentioned, and we have not observed any association with the local Dominion Astrophysical Observatory seismograms.

Figs. 1 and 2 show the records at our base station near Victoria, B.C., for the two dates referred to above. The records for $\delta X/\delta t$, $\delta Y/\delta t$ and $\delta Z/\delta t$ are shown. Full scale on each record is about 1 gamma for the longer-period fluctuation. Fig. 3 shows the simultaneous records of $\delta X/\delta t$ at our base station and of $\delta Z/\delta t$ near Borrego Springs about 1,100 miles away in southern California.

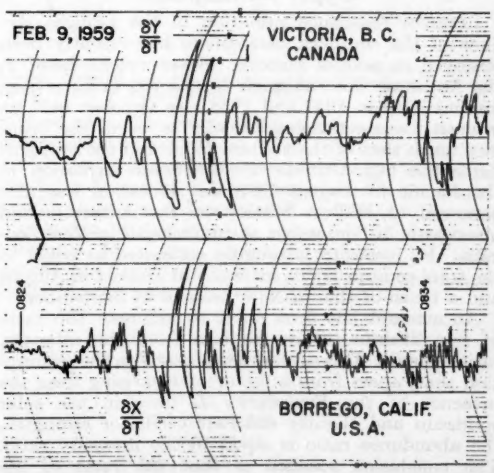


Fig. 3. Records of February 9, 1959, between 0821 and 0836 G.M.T. of $\delta Y/\delta t$ taken at Albert Head base station, near Victoria, B.C., Canada, and of $\delta Z/\delta t$ taken at Borrego, California, U.S.A.

It is probable that these fluctuations are associated with the 'oscillatory bursts' in earth-current records examined by V. A. Troyiçkaya. These, she states, are persistently associated with characteristic (magnetic) oscillations having a period of the order of 2–3 sec.[3].

The longer-period damped fluctuations are of interest in another respect. Examination of the magnetograms of the local Dominion Observatory, of which Mr. Bernard Caner has kindly supplied copies, shows that these damped fluctuations can often be recognized on the magnetograph records as a very sudden and small displacement of *H*, *D* and *V* (from 1 gamma upwards). While these displacements may be seen by looking for them at the times suggested by the fluctuation records, the reverse procedure is not very effective.

It is difficult and costly for us to record the geomagnetic fluctuations at far distant places when we are dependent on transport of heavy equipment. Thus we have not operated simultaneously a pair of stations much more than 1,000 miles apart. We believe, however, that this type of fluctuation, which is most obvious in the early part of the night when the general activity is low, may be shown on the magnetograms of observatories covering a large area. We think this examination should be made and will willingly supply dates and times when these fluctuations have been recorded at our base station within the past few months.

This work is supported by the Defence Research Board of Canada and is part of the programme of the Pacific Naval Laboratory.

H. J. Duffus
J. A. Shand
Charles Wright

Pacific Naval Laboratory,
Esquimalt, B.C.

[1] Daniels, Fred B., *Nature*, **182**, 1439 (1958).
[2] Duffus, H. J., Nasmyth, P. W., Shand, J. A., and Wright, Sir Charles, *Nature*, **181**, 1258 (1958).
[3] Troyiçkaya, V. A., *Dok. Akad. Nauk, U.S.S.R.*, **91**, 2, 241 (1953). Translation *T174R*, 6, by Hope, E. R., Defence Research Board, Ottawa.

Origin of Sodium and Lithium in the Upper Atmosphere

Lithium resonance radiation in the twilight airglow of the southern hemisphere has recently been observed in several places[1,2]. Over Adelie Land in the Antarctic this radiation is reported to have been present both in 1957 and 1958. In October 1957 its intensity was estimated visually to be of the order of a tenth that of the sodium *D* lines in the twilight. After the high-altitude nuclear bomb explosion in the Pacific in August 1958 this radiation was also observed at Hallett Station in the Antarctic and afterwards in September at Invercargill in New Zealand. Its intensity is roughly estimated as equal to the total sodium *D* line intensity at Hallett in August and a tenth of this in New Zealand in September.

An attempt has been made to estimate the ratio of free lithium to free sodium from the intensity ratios found[2]. This estimate was based on the assumption that, apart from a factor of 17 arising from the presence of the Fraunhofer *D* lines in the solar spectrum and slightly different oscillator strengths, the abundance ratio is equal to the intensity ratio. The conclusion reached is that the ratio of the abundance of lithium to sodium is of the order of 0·006 (perhaps even 0·02 if ions are also considered). Compared with a ratio of 0·002 expected if the common origin of these elements in the Earth's atmosphere is meteoric debris, and a ratio of $2{\cdot}3 \times 10^{-5}$ expected if their origin is in sea water, the result is taken as strong evidence for the meteoric source.

Similarly, from the New Zealand and Hallett observations an attempt has been made to estimate the amount of lithium in the atmosphere, again on the assumption that sodium and lithium intensities in the twilight airglow are proportional to their abundances[2]. This calculation takes an old estimate of 6×10^9 atoms/cm.^{2} for the abundance of sodium and suggests that the quantity of lithium in the upper atmosphere fell from 20 kgm. to 2 kgm. between August 5 and September 5.

Because of resonance absorption of sunlight in the layers, the assumption of linearity is a very poor one, however, at least in the case of sodium[4]. In fact, the increase of intensity of scattering with abundance becomes significantly less than linear for abundance of sodium in excess of 10^9 atoms/cm.^{2}. In the neighbourhood of 25×10^9 atoms/cm.^{2}, the intensity actually passes through a maximum and declines very slowly for larger quantities of sodium. This non-linearity makes any estimate of the lithium–sodium abundance ratio impossible from the intensity ratios alone. An absolute determination of the quantity of one or the other present is also necessary. Even then the double value in the intensity abundance curve may leave the situation ambiguous.

The season for which the lithium observations are reported was late winter. This is the very time when the seasonal maximum in sodium abundance would be expected. In the northern hemisphere there occurs a sodium maximum at the inland station of Saskatoon in Canada of about 10×10^9 atoms/cm.^{2} in late February. At Haute-Provence Observatory in southern France intensity peaks corresponding to abundances in the neighbourhood of 25×10^9 atoms/cm.^{2} are reached in December and March. Between these intensity maxima lies a minimum in February with which abundances as low as 5×10^9 atoms/cm.^{2} and higher than 100×10^9 atoms/cm.^{2} are consistent because of the maximum in the abundance-intensity curve.

If the sodium in the southern hemisphere behaves similarly, and particularly if the seasonal variation is like that observed in France, sodium abundances from 5×10^9 atoms/cm.^{2} to more than 100×10^9 atoms/cm.^{2} cannot be excluded in August. For this range of abundance the variation of intensity is so small that any quantity of sodium between these limits would be consistent with $3/17 \times 10^8$ atoms of lithium/cm.^{2} and an intensity ratio of the order of 0·1. From the measurements available, therefore, it is safe to conclude only that the lithium–sodium ratio lay somewhere between 0·006 and 2×10^{-5} or, more probably, between 0·0035 and 0·0006.

Of course, the Johnson Island nuclear explosion on August 1, 1958, may very well be the source of at least part of the lithium observed[5]. Lithium–sodium intensity measurements as a consequence have become even less useful in solving the problem of the origin of the atmospheric sodium.

On this question there are three pieces of evidence, none of them conclusive. There were the repeated observations from southern France during 1955 and 1956 of much more sodium towards the Atlantic Ocean than towards the Continent[4]. There is a remarkably different seasonal variation in sodium near the ocean than is found far inland, with much more

sodium near the ocean[6]. Finally, there is the recent attempt by Lytle and Hunten[7] to detect the resonance lines of potassium in twilight. This places an upper limit of 1 : 40 on the potassium–sodium ratio, whereas sea water has a ratio of 1 : 47 and all other sources between 1 : 10 and 1 : 5. At this time the evidence must be taken as favouring a marine origin.

The work reported here was supported in part by a grant from the National Science Foundation.

T. M. Donahue

Department of Physics,
University of Pittsburgh,
Pittsburgh 13, Pennsylvania.

April 20.

[1] Delannoy, J., and Weill, G., *C.R. Acad. Sci., Paris*, **247**, 806 (1958).
[2] Gadsden, M., and Salmon, K., *Nature*, **182**, 1598 (1958).
[3] Barbier, D., Delannoy, J., and Weill, G., *C.R. Acad. Sci., Paris*, **247**, 886 (1958).
[4] The consequences of this fact have been treated in a series of papers by Blamont and Donahue, Chamberlain and Hunten and by Galperin and their collaborators. References may be found in Blamont, J. E., Donahue, T. M., and Stull, V. R., *Ann. Geophys.*, **14**, 253 (1958); Chamberlain, J. W., Hunten, D. M., and Mack, J. E., *J. Atm. and Terr. Phys.*, **12**, 153 (1958) and Galperin, G. I., "The Airglow and the Aurorae", 95, edit. by Armstrong and Dalgarno (Pergamon Press, London, 1956).
[5] Barber, D. E., *Nature*, **183**, 384 (1959).
[6] Blamont, J. E., and Donahue, T., *C.R. Acad. Sci., Paris*, **247**, 496 (1958).
[7] Lytle, E. A., and Hunten, D. M., *J. Atm. and Terr. Phys.* (in the press); I wish to thank Dr. Hunten for an opportunity to see his results before publication.

Effect of Cathode Inclusions on Electrical Discharges

Recognition has been given for some considerable time now to the fact that the surface of the negative electrode in a spark gap can influence the electrical discharge[1]. An attempt has been made to ascertain the location of the starting place of arc spots in relation to the metallurgical features of the cathodes.

As is well known, metals frequently contain microscopically visible inclusions often of more than one type chemically. In stainless steels, with which we have been principally concerned, oxides, nitrides, carbides, slag particles and pieces of refractories are the major inclusions.

Microscopical examination of clean polished specimens which had been used as negative electrodes in a small experimental spark gap showed that metal had vapourized from around certain inclusions, the bulk of the material being unaffected. It is of considerable interest to note that the inclusions affected possessed low electrical conductivity whereas inclusions of relatively good conductivity were entirely unaffected.

This phenomenon is by no means peculiar to stainless steels. For example, localized damage has been found around cuprous oxide inclusions in samples of copper. Cuprous oxide also has a low electrical conductivity. The current passing in these experiments under non-arcing conditions was approximately $1{\cdot}6 \times 10^{-4}$ amp. cm.$^{-2}$ and the minimum resistivity for arc initiation appears to be 10^3 ohm. cm.

This work, which forms part of a programme to investigate surface properties of metals, is to be reported in detail elsewhere.

P. C. L. Pfeil
L. B. Griffiths

Metallurgy Division,
Atomic Energy Research Establishment,
Harwell, Berks.

April 3.

[1] See, for example, Jones, F. Ll., "The Physics of Electrical Contacts" (Oxford Univ. Press, 1957).

X-ray Spectrographic Determination of Strontium

Roberts[1] recently described an X-ray fluorescence spectrographic method for the determination of strontium in animal bone using the Philips *PW* 1520 spectrograph fitted with a quartz analysing crystal. In our laboratories a lithium fluoride crystal was available and was used for similar analyses since, compared with a quartz crystal, it increases the intensity of the strontium $K\alpha$ line by a factor of 2·8 and the line/background ratio by a factor of 1·15. Thus the counting time to achieve a given coefficient of variation (due to the counting statistics) can be reduced by a factor of 3·5 or, if the same counting time is compared, the coefficient of variation can be reduced by a factor of 1·9, by using the lithium fluoride crystal.

The instrumental conditions were: Philips X-ray spectrograph, *PW* 1520; tungsten target tube, 48 kV., 20 m.amp.; argon-filled Geiger counter; X-ray path evacuated; exit collimator not fitted. Samples were prepared in a manner similar to that described by Roberts, but the 'Mylar' film was not removed from the sample holder because of the potential danger of contamination of the window of the X-ray tube. The absorption of the strontium radiation by this film is theoretically about 0·1 per cent and, in practice, was not detected.

The specific intensity of the strontium $K\alpha$ line from milk powder ash was found to be identical with that from animal bone ash, and results obtained by several different methods for both these types of sample are compared in Table 1.

Table 1

Sample	Strontium content (p.p.m.)			
	X-ray spectrography	Emission spectrography (ref. 2)	Neutron activation*	Flame photometry (ref. 2)
Bone ash				
1	90	90	89	89
2	120	109	—	124
3	198	190	—	192
4	340	345	—	335
Milk ash				
1	33	36	33	32
2	34	31	32	33
3	56	45	53	51
4	65	64	65	64

* Determinations carried out by Dr. G. E. Harrison and Mrs. E. Sowden, Medical Research Council Radiobiological Unit, Atomic Energy Research Establishment, Harwell.

Since these determinations were carried out, a molybdenum-target X-ray tube has been obtained and shown to increase the intensity of the strontium line by a factor of 1·5 and improve the line/background ratio by a factor of 2·5. Thus with this tube and a lithium fluoride crystal it is possible to determine 10 p.p.m. strontium in bone or milk powder ashes in 30 min. with a coefficient of variation due to the counting statistics of 10 per cent.

J. T. Campbell
H. I. Shalgosky

U.K. Atomic Energy Authority (Research Group),
Chemistry Division,
Woolwich Outstation,
C.37 Royal Arsenal,
Woolwich,
London, S.E.18. April 4.

[1] Roberts, W. M. B., *Nature*, **183**, 887 (1959).
[2] Jury, R. V., Webb, M. S. W., and Webb, R. J., U.K. Atomic Energy Authority Report No. *C/R* 2614 (Nov. 1958).

FORTHCOMING EVENTS

*(Meetings marked with an asterisk * are open to the public)*

Tuesday, May 26

University of London (at Imperial College of Science and Technology, South Kensington, London, S.W.7), at 5.30 p.m.—Prof. W. O. James, F.R.S.: "Botany—Here and Now".* (Inaugural Lecture.)

University of London (at the Institute of Archæology, Inner Circle, Regent's Park, London, N.W.1), at 6 p.m.—Mr. A. F. Shore: "Animals in Ancient Egypt".* (Fourth of six lectures on "Animals in Archæology". Further lectures on June 2 and 9.)

Tuesday, May 26—Friday, June 5

National Union of Teachers (at the National Hall, Olympia, London)—National Education and Careers Exhibition.*

Wednesday, May 27

Royal Statistical Society (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5 p.m.—Dr. J. Kiefer: "Optimum Experimental Designs".

Society of Chemical Industry, Food Group (at 14 Belgrave Square, London, S.W.1), at 6.15 p.m.—Annual General Meeting, followed by Dr. J. R. Nicholls: "Food and Ordinary Man".

Jephcott Lectures Trust (at the Royal Society of Medicine, 1 Wimpole Street, London, W.1), at 7 p.m.—Sir Alexander R. Todd, F.R.S.: "Nucleic Acids and their Role in Future Chemotherapy of Tumours and Virus Diseases" (First Jephcott Lecture).

British Psychological Society, Medical Section (in the House of the Royal Society of Medicine, 1 Wimpole Street, London, W.1), at 8.30 p.m.—Dr. Henri Rey: "Studies on the Relationship of Hormones, Brain Activity and Behaviour in Women".

Royal Institution (at 21 Albemarle Street, London, W.1), at 9 p.m.—Prof. Sir Harold Spencer Jones, F.R.S.: "Some Principles of Clock and Watch Design".

Thursday, May 28

University of London (at the Postgraduate Medical School of London, Ducane Road, London, W.12), at 4 p.m.—Dr. E. E. Pochin: "The Therapeutic Use of Radio-iodine".*

Royal Society (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Mr. R. P. Bell, F.R.S., and Mr. T. Spencer: "Kinetics of the Base-Catalysed Bromination of Ethyl Nitroacetate"; Mr. C. F. Cullis, Mr. F. R. F. Hardy and Mr. D. W. Turner: "The Point of Oxygen Attack in the Combustion of Hydrocarbons. II: The Formation and Origin of Ketones".

Institute of Metals (at 17 Belgrave Square, London, S.W.1), at 6.30 p.m.—Prof. N. J. Petch: "The Atomic Mechanisms of Fracture".

Friday, May 29

University College, Department of Mathematics (in the Eugenics Theatre, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. R. Thom (University of Strasbourg): "General Position and Transversality".*

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Lecturer in Civil Engineering—The Registrar, The University, Sheffield (May 30).

Lecturer (with a university degree, and preferably previous forensic experience) in Forensic Chemistry in the Department of Forensic Medicine—The Secretary, The University, Edinburgh (May 30).

Map Curator and Cartographer (graduate in geography with a special interest in cartography and map preparation) in the Department of Geography—The Registrar, University College, Singleton Park, Swansea (May 30).

Demonstrator (with a good honours degree in physiology, biochemistry or chemistry) in Chemical Physiology—The Registrar, The University, Manchester 13 (June 1).

Assistant Librarian (with a university degree)—The Secretary, The University, Aberdeen (June 5).

Lecturer (specialized in parasitology) in the Department of Zoology—The Registrar, The University, Hull (June 6).

Assistant Lecturer (preferably with a knowledge of embryology or vertebrates) in the Department of Zoology—The Registrar, University College of Wales, Aberystwyth (June 12).

Lecturer or Assistant Lecturer in Electronics—The Secretary and Registrar, The University, Southampton (June 12).

Electron Microscopist (graduate in medicine, biology or physics, and experienced in the operation and maintenance of a high-resolution instrument (preferably Siemens) and in the techniques of specimen preparation) in the Department of Physiology and Histology—The Registrar, The University, Liverpool (June 13).

Lecturer and an Assistant Lecturer in Physics—The Registrar, The University, Hull (June 13).

Demonstrator (with a degree in agriculture or an honours degree in pure science followed by an adequate postgraduate qualification in agriculture, and preferably some training in field experimentation)—The Secretary, School of Agriculture, The University, Downing Street, Cambridge (June 15).

Scientific Officer (with a good honours degree in botany and preferably experience in plant physiological research) in the Botany Department, for work on the physiology of the sugar beet plant, especially on the causes of variation in yield of field crops—The Secretary, Rothamsted Experimental Station, Harpenden, Herts (June 15).

Electronic Computing Engineer (university graduate experienced in the development and design of electronic computing equipment) on the staff of the Adolph Basser Computing Laboratory in the School of Physics, to assume responsibility for a part of the development programme—The Registrar, University of Sydney, Sydney, N.S.W., Australia (June 19).

Assistant Experimental Officer (with a degree in a biological subject and practical experience in bacteriological methods and techniques), for work on the ecology of soil micro-organisms—The Secretary, Rothamsted Experimental Station, Harpenden, Herts (June 30).

Botanist (with an honours degree in botany from a recognized university and preferably at least two years postgraduate experience), for duties particularly concerned with the study of the growth characteristics of the tea bush in the chief soil-climatic regions of East Africa, the study and provision of improved planting material, and the botanical interpretation of field experiments—The Director, Tea Research Institute of East Africa, P.O. Box 91, Kericho, Kenya Colony (June 30).

Research Assistants (with an honours degree of a British university or equivalent qualification) in Plant Biochemistry, Electrical Engineering, Applied Physics, Mechanical Engineering, Applied Chemistry, Mining, and Biochemical Microbiology—The Principal, Heriot-Watt College, Edinburgh (June 30).

Soil Scientist (with a Ph.D. degree in soil chemistry and preferably with some experience), to carry out soil fertility investigations, both applied and fundamental, in relation to tobacco, and to supervise soil analysis—The Director, Tobacco Research Board of Rhodesia and Nyasaland, P.O. Box 1909, Salisbury, Southern Rhodesia (June 30).

Assistant Lecturer (Grade B) in Chemistry—The Principal, Peel Park Technical College, Salford 5.

Chair of Engineering—The Registrar, The University, Leicester.

Head (with high academic qualifications, and experience of full-time teaching, together with industrial and/or research experience) of Physics and Mathematics Department (Grade IV)—The Registrar, Portsmouth College of Technology, Portsmouth.

Lecturer in Forestry—The Principal, Royal Agricultural College, Cirencester.

Physicist, Basic grade (with a first- or second-class honours degree), for routine and development work in hospital physics—The Secretary, Lambeth Hospital, Brook Drive, London, S.E.11.

Plant Pathologist (with a good honours degree in botany or plant pathology and approved postgraduate training) in Kenya, for laboratory and field research work on the identification and control of plant diseases—The Director of Recruitment, Colonial Office, London, S.W.1, quoting BCD.63/7/030.

Plant Pathologist (with a good honours degree in botany and with two years postgraduate experience in plant pathology) in the Department of Agricultural Research, Federation of Nigeria, for research into virus diseases of food crops—The Director of Recruitment, Colonial Office, London, S.W.1, quoting BCD.63/14/035.

Special Officer (Statistician) (with a good honours degree, preferably in agriculture, with at least two years suitable postgraduate training or experience in statistics) in the Northern Region of Nigeria, to advise on the design and supervise analysis and presentation of results of experimental work for all sections of the Research Branch of the Northern Region Department of Agriculture, and to take part in the development of satisfactory experimental techniques in the field—The Director of Recruitment, Colonial Office, London, S.W.1, quoting BCD.63/408/032.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Association of Universities of the British Commonwealth. Report of the Executive Council together with the Accounts of the Association for the year 1st August, 1957 to 31st July, 1958. Pp. 25. (London: Association of Universities of the British Commonwealth, 1959.) [272

Ciba (A.R.L.), Ltd. Technical Notes No. 194 (February, 1959): Aerolite in Schools and Colleges. Pp. 8. (Duxford: Ciba (A.R.L.), Ltd., 1959.) [272

The Carnegie Trust for the Universities of Scotland. Fifty-seventh Annual Report for the year 1957–58, submitted by the Executive Committee to the Trustees on 23rd February 1959. Pp. iv+74. (Edinburgh: Carnegie Trust for the Universities of Scotland, 1959.) [272

Editorial and Publishing Offices of "NATURE"
MACMILLAN & CO., LTD.,
ST. MARTIN'S STREET, LONDON, W.C.2.
Telephone Number: Whitehall 8831. Telegrams: Phusis Lesquare London

Annual subscription £7/15/–, payable in advance, postage paid to any part of the world

Advertisements only should be addressed to
T. G. Scott & Son, Ltd., 1 Clement's Inn, London, W.C.2.
Telephone Number: Holborn 4743

All rights reserved. Registered as a newspaper at the General Post Office